**Advances in Biochemistry**
2024; 12(1): 10-19
http://www.sciencepublishinggroup.com/j/ab
doi: 10.11648/j.ab.20241201.12
ISSN: 2329-0870 (Print); ISSN: 2329-0862 (Online)

# *In Vitro* Antioxidant Properties and Interleukin-17 Expression Levels of *Eribroma oblongum* (Malvaceae) in Wistar Rats with Atherogenic Diet-Induced Steatotic Liver

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## To cite this article:

Tsague Marthe Valentine, Njiki Bikoï Jacky, Sokamte Tegang Alphonse, Edoun Ebouel Ferdinand Lanvin, Nodem Sohanang Francky Steve et al. (2024). *In Vitro* Antioxidant Properties and Interleukin-17 Expression Levels of *Eribroma oblongum* (Malvaceae) in Wistar Rats with Atherogenic Diet-Induced Steatotic Liver. *Advances in Biochemistry*, *12*(1), 10-19. https://doi.org/10.11648/j.ab.20241201.12

**Received**: December 11, 2023; **Accepted**: January 11, 2024; **Published**: January 23, 2024

**Abstract:** Introduction: *Eribroma oblongum* (Malvaceae) (EO) stem bark is also used in traditional medicine to treat cardiovascular diseases and painful menstruation. This study aimed to estimate the mineral composition, *in vitro* antioxidant properties, and Interleukin-17 (IL-17) expression levels of hydroethanolic extract (EOHE) and the powder fraction 125µm (EOP) of *Eribroma oblongum* (EO), in Wistar rats with atherogenic diet-induced steatotic liver. Materials and methods: The mineral composition of these therapeutic plants was characterized through atomic absorption spectrometry. Antioxidant activities were measured using spectroscopic methods. Rats were given daily doses of EOP, EOHE (100mg/kg), or Atorvastatin (10mg/kg) as a positive control for 45 days at the same time as being fed an atherogenic diet. At the end of the treatment period, animals were sacrificed and liver and blood were collected for evaluation of biochemical parameters. The serum levels of cytokines IL-17 were measured by ELISA. Results: The results showed that EOP had a significantly $P\ 0.0001$ higher composition of Iron (25.75±0.6464g/100g DM), Zinc (11.71±7164g/100g DM), Vitamin C (41.60 ± 4.864g/100g DM), Manganese (2.02±0.0164g/100g DM), Selenium (0.037±0.000764g/100g DM) than EOHE. EOHE had a significantly higher polyphenol content ($p<0.05$) than EOP 917.33±23.63µg CaE/g DM. EOP and EOHE had good polyphenol contents (in descending order EOHE (995.43±24.10 (µg CaE/g DM)) > EOP(917.33±23.63 (µg CaE/g DM)). DPPH, FRAP, and TAC increased positively with hydroethanolic extract and powder concentration. EOP significantly ($P\ 0.0001$) reduced IL-17 expression by 48,76% compared with the negative control group. Conclusion: These results demonstrated that EOP can be a source of anti-inflammatory agents in the fight against metabolic diseases. The therapeutic potential of *Eribroma oblongum* for the treatment of steatosis could be considered in further studies.

**Keywords:** *Eribroma oblongum*, Mineral Composition, Antioxidant Properties, Expression of Interleukin-17

## 1. Introduction

Medicinal plants have always been an important source for phytomedicinal development. Phytomedicine studies have become an important priority for the World Health Organization, as almost 80% of the population in developing

countries use these plants for the treatment of many diseases [1, 2]. The search for new natural molecules of biological interest with antioxidant properties is currently a major focus of the pharmaceutical and agri-food industries [3]. These bioactive compounds have good antioxidant potential and a range of biological properties, including anti-hypertensive, and above all anti-inflammatory properties [4]. These natural molecules can be found in plants and are characterized by important biological properties, and an ethnobotanical knowledge of these properties can form a very good basis for the development of new therapeutic strategies [5]. A diet high in saturated fatty acids can lead to non-alcoholic steatosis hepatitis, which is associated with metabolic liver diseases involving steatosis and inflammation [6]. Interleukin 17 (IL-17) is a recently discovered pro-inflammatory cytokine that plays a role in various inflammatory diseases such as psoriasis, psoriatic arthritis, rheumatoid arthritis, and ankylosing spondylitis [7]. IL-17 stimulates the infiltration of immune cells and causes liver damage, leading to hepatic inflammation and fibrosis [8]. It is also implicated in autoimmune liver diseases. Elevated levels of circulating IL-17 and increased frequency of IL-17-producing cells have been observed in both acute and chronic liver diseases. Studies focusing on the effects of deleting or neutralizing IL-17 in various murine models have shown beneficial effects, suggesting that targeting the IL-17 axis could be a promising therapeutic strategy to prevent the chronicity and progression of different liver diseases [8].

This study aimed to assess the mineral composition, *in vitro* antioxidant properties, and Interleukin-17 expression levels of the powder fraction < 125µm and hydroethanolic extract of *Eribroma oblongum* in Wistar rats with atherogenic diet-induced steatotic liver.

# 2. Materials and Methods

### *2.1. Plant Material*

The specimens for this study consisted of the stem bark of *Eribroma oblongum* (Malvaceae) harvested in September 2021 in the village of Edea, Littoral Region, and registered at the National Herbarium (HNC) in Yaounde-Cameroon where a reference specimen N°27489SRFCam) [9].

### *2.2. Powder Production Process*

The BIOBASE electric pulverizer (model MPD-102, N°: 61 South Gongye Road Jinan City, China; serial number 20020020) equipped with a 1 mm trapezoidal hole mesh was used to grind the materials. Grinding was performed at 1400 rpm for 1 minute in the atmosphere [10].

Samples were sieved with a laboratory electric sieve (MINOR) according to their particle size. The process of sieving is based on the separation of particles through several sieves of decreasing mesh size. 250 g of sample was sieved through a mesh (125 µm) and placed on the sieve, then agitated by vertical vibrations of 0.5 mm in magnitude for 30 minutes. The powder obtained was then weighed. This enabled us to produce a powder fraction particle size class < 125 µm. These powder fractions were then conditioned in polyethylene containers and stored at 4°C for later research [11]. The mass of the fraction (%) was calculated and defined as the ratio between the mass of dry matter sieved on each sieve and the weight of the total sieved powder. The mass of the fraction was computed according to the following equation (1):

$$Fraction\ mass\ (\%) = \frac{Weight\ of\ each\ powder\ fraction}{Weight\ of\ total\ sieved\ powder} x\ 100 \quad (1)$$

### *2.3. Process for Obtaining a Hydroethanolic Extract of Eribroma Oblongum*

680g of powder were macerated in 1L of a mixture of water and ethanol (50:50 v/v). for *E. oblongum* stem bark for 48 hours. The resulting macerate was filtered through Whatman N°4 filter paper and the filtrate was concentrated using a rotavapor. This operation, replicated many times, yielded 90 g of concentrated crude extract in the form of a brown oily paste representing EOHE, giving an extract yield of 13.23%.

### *2.4. Determination of Some Minerals*

Mineral analysis is carried out by incinerating the sample powders in a muffle furnace at 550°C. All these minerals were extracted after dissolving the ashes (1g) in 10 mL of hydrochloric acid (1.5 N), and then the mixture was completely dried on a hot plate. Then, a few drops of $H_2O_2$ and 5 mL of deionized water are added and made up to 25 mL in a calibrated glass flask. These solutions obtained are repeated in triplicate of Iron (Fe), Selenium (Se), Copper (Cu), Calcium (Ca), Zinc (Zn), Vitamin C (Vit C), and Manganese (Mn) readings made by Atomic Absorption Spectrophotometry (AAS) (Hitachi, Tokyo, Japan) [10].

### *2.5. Powder Sample Analysis Process*

Moisture and ash content were determined using the official AOAC method [12]. The experiment was carried out in three replicates.

### *2.6. Determination of Phenolic Compounds*

The objective of the quantitative study of the samples using spectrophotometric assays was to determine the total content of total polyphenols, and flavonoids. Three calibration curves were plotted for this purpose and carried out for each type of assay. The results in Catechin Equivalent and Quercetin Equivalent respectively, the results are expressed in µg/g of dry matter.

#### *2.6.1. Determination of Total Polyphenols Compounds*

The total phenolic content of the dry samples was determined with the Folin-Ciocalteu reagent [13]. The results are expressed as micrograms of gallic acid (GA) equivalent (E) per gram of dry matter of extract (µg GAE/g DM). All measurements were repeated 3 times.

### *2.6.2. Determination of Total Flavonoids*

The total flavonoid content was determined with an aluminum chloride colorimetric assay [14]. The data of flavonoid content was expressed in micrograms of quercetin (Q) equivalents (E) per gram of dry matter (µg QE/g DM), all samples were reproduced at least three times under the same conditions.

### *2.7. Determination of in Vitro Antioxidant Activities*

### *2.7.1. Ferric Reducing Antioxidant Power*

Principle The FRAP process is based on the reduction of ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$). This procedure is used to measure the reducing power of compounds [15]. The use of reducing agents in plant extracts leads to the reduction of the $Fe^{3+}$/ferricyanide complex to the ferrous form. As a result, $Fe^{2+}$ can be quantified by measuring and monitoring the increase in cyan-blue color density in the test medium at 700 nm [16]. The $FeCl_3/K_3Fe(CN)_6$ system gives the method sensitivity for the "semi-quantitative" determination of antioxidant concentrations, which are involved in the redox reaction [17].

The assay method the protocol used in the laboratory are based on that described by Oyaizu [18]. In a glass test tube containing 200 µL of sample solution at different concentrations, 500 µL of phosphate buffer (0.2M: pH 6.6) was added, followed by 500 µL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] 1% in distilled water. The whole mixture was heated to 50°C in a water bath for 20 minutes. A volume of 500 µL of trichloroacetic acid (10%) was then added and the mixture was centrifuged at 3000 rpm for 10 minutes. A 500 µL aliquot of the supernatant was transferred to another tube to which 500 µL of distilled water and 100 µL of freshly prepared 1% $FeCl_3$ in distilled water had been added. A blank without a sample was prepared under the same conditions, replacing the extract with methanol. The absorbance of the reaction mixture was read at 700 nm versus a blank prepared similarly, replacing the extract with methanol to calibrate the instrument (UV/Visible spectrophotometer). The control was a solution of a standard antioxidant, ascorbic acid, whose absorbance was determined under the same experimental conditions as the samples. An increased absorbance indicates an increase in the reducing power of the extracts measured.

### *2.7.2. DPPH Radical Scavenging Activity*

Antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, which measures the ability of the hydroethanolic extract and the powder fraction < 125 µm EO to donate hydrogen atoms or electrons. 0.5 mL of a sample of the EOHE/EOP at different concentrations (1, 2, 3, and 4 mg/mL) or ascorbic acid (employed as a reference at different concentrations (0.025, 0.05, 0.1, 0.5, and 1 mg/mL)) will react with 2 mL of 0.1 mM DPPH methanolic solution in the dark for 1 h at room temperature, then the absorbance (Abs) will be measured at 517 nm using Ultraviolet/Visible spectrophotometry. The radical scavenging activity was estimated as a percentage of DPPH discoloration using the Equation 2 [10, 14]:

$$\text{DPPH activity (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}}\ x\ 100 \qquad (2)$$

where $Abs_{control}$ is the absorbance of the control; $Abs_{sample}$ is the absorbance of the sample

$IC_{50}$ is the inhibitory concentration of crude extract or powder fraction < 125µm capable of trapping 50% of the DPPH radical or inhibiting 50% oxidation. $IC_{50}$ is determined by plotting the percentage of inhibition against the concentration of extract or powder fraction < 125µm. $IC_{50}$ value is inversely related to the activity and a lower $IC_{50}$ value means higher antioxidant activity. We have the equations of lines (3) and (4).

$$Y_{(EOHE)} = 35.446\ \ln(x) + 3.136;\ R^2 = 0.9005 \qquad (3)$$

$$Y_{(EOP)} = 25.464\ \ln(x) - 6.4754;\ R^2 = 0.7272 \qquad (4)$$

Where R is the slope

### *2.7.3. Determination of Total Antioxidant Capacity "Phosphomolybdate Test"*

The PhosphoMolybdate test (PPM) is a development of the DPPH test. In this reaction, hydrogen and electrons are transferred from the reducing compound (extract-antioxidant) to the oxidizing complex (PPM). This transfer depends on the redox potential, the pH of the medium, and the structure of the antioxidant compound.

Principle Molybdenum Mo (VI) in the form of molybdate ions $MoO4^{2-}$ was tested for its ability to reduce to molybdenum Mo (V) $MoO^{2+}$ in the presence of an antioxidant agent or extract. At an acidic pH, this reduction manifests as the creation of a greenish complex (phosphate/Mo(V)) [19]. When antioxidants are present, the molybdenum (VI) complex's increased color is measured.

The procedure includes filling the tube with 200 µL of each extract at various concentrations. Concentrations combined with 2000 µL of a reagent containing 4 mM ammonium molybdate, 28 mM $Na_2PO_4$, and 0.6 M $H_2SO_4$. After that, the tube was securely closed and incubated for 90 minutes at 95°C. At 695 nm, absorbance was measured following cooling. 2000 µL of the previously described reagent was combined with 200 µL of methanol used as the control.

The same conditions were used for the incubation of the samples and controls. The acquired values are reported in milligrams (mg AAE/g DM) of ascorbic acid equivalent per gram of extract dry matter extract.

### *2.8. Animal Study*

The male animals included in our research weighed approximately 200 grams. All of them were given to us by the animal facility of the Laboratory of Biophysics, Food Biochemistry and Nutrition of ENSAI, University of Ngaoundere, with 12 hours of light and 12 hours of darkness, and an ambient temperature of about 20–25±1°C. *Ad libitum* access to drinking water was provided. These healthy animals

were allotted at random to the various groups. Not one of the animals had ever been the focus of an experiment before. The Cameroonian National Ethics Committee gave its clearance for this work, ref. no. FWIRD00001954 [20].

#### *2.8.1. Treatment Design*

The experiment was conducted on 28 male Wistar rats. After acclimation, the rats were randomly divided into the following groups with 4 rats in each group and per cage:

Group 1: Control or normal group: received distilled water (1mL/100g bw/day, *per os*) and was fed the normal diet;

Group 2: The negative control group received distilled water (1mL/100g bw/day, *per os*) and was fed the atherogenic diet;

Group 3: The positive control group received atorvastatin (10mg/kg bw/day, *per os*) and was fed the atherogenic diet;

Group 4: received the hydroethanolic extract of *Eribroma oblongum* (100mg/kg bw/day, *per os*), and were fed the atherogenic diet;

Group 5: The test group received the powder of *Eribroma oblongum* (100mg/kg bw/day, *per os*), and were fed an atherogenic diet.

Each powder or extract sample was first dissolved in distilled water by churning with a magnetic stirrer set to 3500 rpm for 12 hours before the rats were fed the chow. For 45 days, macerated samples (for the experimental rat groups) and distilled water (for the negative control and normal control rat groups) were administered daily. Animals were fasted overnight without dewatering. Then the rats were anesthetized with Ketamine/ Valium (0.2/0.1 mL/100g bw in IP). Arterial blood was collected in dry tubes. Blood and homogenates were centrifuged at 2500 rpm for 15 minutes. The supernatant will be collected and stored at - 20°C for biochemical analyses. Part of the liver was used to make the histological sections.

***Table 1.*** *Composition of the normal diet and the atherogenic diet was modified [9].*

| Composition | Normal diet (%) | Atherogenic diet (%) |
|---|---|---|
| Protein: fish powder | 12 | 10 |
| Carbohydrates: 50% pulped corn flour + 50% wheat flour | 71 | 61 |
| Sugar: table sugar | 05 | 05 |
| Fatty acid: lard | 05 | 16 |
| Salts: cooking salts 3% + Calcium 1% | 04 | 04 |
| Fiber: cellulose | 02 | 01 |
| Cholesterol: cooked egg yolk | - | 01 |
| Vitamins | 01 | 02 |
| Total weight (g) | 100 | 100 |

#### *2.8.2. Biochemical Analysis*

*Measurement of Interleukine-17 in Blood Serum*

The IL-17 assay was performed using a RayBiotech ELISA kit, and optical densities were read using the HumanReader HS automated system.

The principle is based on the specific attachment of an antigen (Ag) to its Antibody. The antigen of interest is trapped between a capture antibody and a detection Ac. An enzymatic complex and a fluorochrome are attached to the detection of Antibodies, enabling the formation of the immune complex to be highlighted.

Label the 8-well strips according to your requirements. Transfer 100 µL of each calibration standard and test sample to the corresponding wells. Apply coverslips and incubate for 2.5 hours at ambient temperature, stirring gently. Throw away the solution and wash it 4 times with 1X Lavage Solution. Wash by filling each well with wash buffer (300 µL) using a pipette or automatic washer. The complete removal of liquid at each stage is essential for good results. After the last wash, remove any residual wash buffer by aspiration or decantation. Turn the plate over and blot on clean absorbent paper. Add 100 µl of prepared 1X biotinylated antibody to each well. Incubate for 1 hour at room temperature, shaking gently. Throw away the solution and repeat the wash. Add 100 µL of prepared streptavidin solution to each well. Incubate for 45 minutes at room temperature, shaking gently. Throw away the solution and repeat washing. Add 100 µL of one-step TMB substrate reagent (element H) to each well. Incubate for 30 minutes at room temperature in the dark, shaking gently. Add 50 µL stop solution (element I) to each well. Read at 450 nm immediately.

### *2.9. Statistical Analysis*

The data were presented as mean ± standard error with three replications per sample. IBM SPSS Statistics version 23 was utilized for analysis. A one-way ANOVA test with one factor (particle size) was employed to assess the presence of statistically significant differences. Tukey's multiple range tests were conducted to determine the significance of the difference between the two means. The level of statistical significance was set at $p < 0.05$.

## 3. Results

Our study first evaluated the content of seven minerals (Iron, Calcium, Zinc, Manganese, Selenium, Copper, and Vitamin C) in the hydroethanolic extract of *Eribroma oblongum* (EOHE), and in powder of *Eribroma oblongum* stem bark (EOP), We then assessed *in vitro* antioxidant activity (DPPH, FRAP, and TAC), and investigated the influence of EOHE and EOP on Interleukin-17 expression in Wistar rats with a steatotic liver.

### *3.1. Mineral Analysis*

A total of seven mineral elements (Copper, Iron, Calcium,

Manganese, Selenium, Zinc, and Vitamin C) were determined in the powder fractions and extracts of *E. oblongum*, as shown in Table 2.

***Table 2.*** *Composition in some minerals of hydroethanolic extract and powder fraction < 125µm of Eribroma oblongum.*

| Constituents (g/100 g DM) | Hydroethanolic extract EOHE | Powder fractions EOP (< 125 µm) |
|---|---|---|
| Dry Matter content | 91.92±0.48 | 84.61±0.47**** |
| Moisture | 8.08±0.48 | 15.39±0.47**** |
| Ash Content | 5.56±0.22 | 6.5±0.07** |
| Iron | 11.95±0.13 | 25.75±0.37**** |
| Calcium | 172.9±2.12 | 211.3±0.00*** |
| Manganese | 1.5±0.05 | 2.02±0.01* |
| Zinc | 9.86±0.08 | 11.71±0.09**** |
| Selenium | 0.028±0.00 | 0.037±0.00** |
| Copper | 2.82±0.04 | 3.75±0.02 |
| Vitamin C | 79.60±0.46 | 41.60±2.77**** |

EOHE: Hydroethanolic Extract of *Eribroma oblongum*; EOP: *Eribroma oblongum* powder fraction < 125 µm; < 125 µm: Powdery fractions with a diameter inferior at 125µm.
Values are expressed as means ± SEM; n=3; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{****}p<0.0001$ significant differences from EOHE

Moisture content was significantly (*P=0.004*) increased in EOP (15.39±0.47g/100g DM) compared to EOHE (8.08±0.48g/100g DM). Ash content was significantly (*P<0.0001*) higher in EOP (6.5±0.04g/100g DM) than in EOHE (5.56±0.12g/100g DM).

Vitamin C, zinc, and iron were significantly (*P<0.0001*) increased in EOP (41.60±2.77, 11.71±0.05, 25.75±0.37g/100g DM) respectively compared to EOHE (79.60±0.46, 9.86±0.04, 11.95±0.13g/100g DM) respectively. Calcium was significantly higher (*P=0.0005*) in EOP (211.3±0.00g/100g DM) than in EOHE (172.9±2.12g/100g DM). Selenium was significantly (P=0.0015) higher in EOP (0.037±0.00g/100g DM) than in EOHE (0.028±0.00g/100g DM). Manganese was significantly (*P=0.024*) higher in EOP (2.02±0.00g/100g DM) than in EOHE (1.5±0.03g/100g DM).

### *3.2. Bioactive Compounds*

Figure 1 shows the data on the compound content of EOP and EOHE. These results show that EOP and EOHE had good polyphenol contents (in descending order EOHE (995.43±24.10 (µg CaE/g DM)) > EOP(917.33±23.63 (µg CaE/g DM)); however, it is important to note that EOHE had a significantly higher polyphenol content (*p<0.05*) than the EOP (917.33±23.63 (µg CaE/g DM)). When we look at flavonoid content, in order, these levels are high in the EOP (109.02±5.01 (µg QE/g DM)) > EOHE (42.46±6.52 (µg QE/g DM)). However, the flavonoid content nevertheless remained significantly (*p<0.05*) high in EOP.

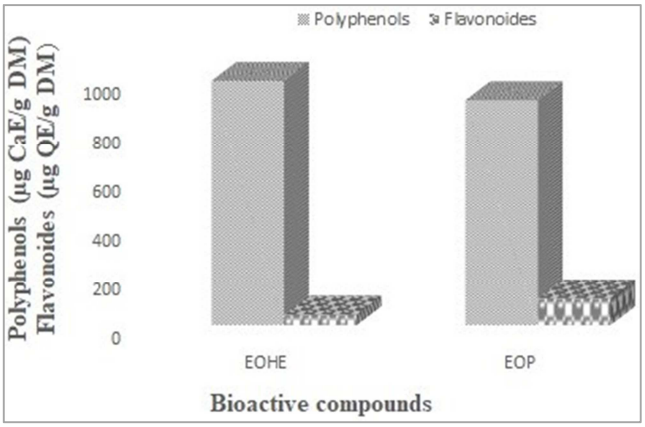


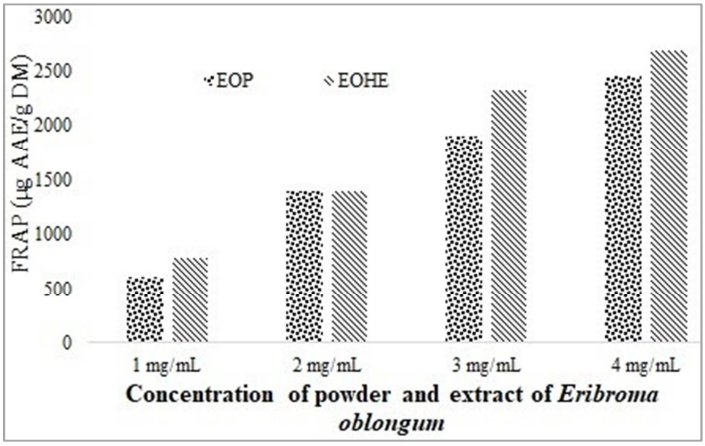

***Figure 1.*** *Contents of bioactive compounds in hydroethanolic extract and powder fraction < 125µm of Eribroma oblongum.*

µg: Microgramme; CaE: Catechin Equivalent; g: Gramme; DM: Dry Matter; QE: Quercetin Equivalent; EQi: Quinine Equivalent; EOHE: hydroethanolic extract of *Eribroma oblongum*; EOP: *Eribroma oblongum* powder fraction < 125µm

### *3.3. Antioxidant Activity*

#### *3.3.1. Ferric Reducing Activity Potential (FRAP)*

Figure 2 below shows the ferric iron reduction capacity of the EOP and EOHE. These data show that, as in the case of DPPH radical scavenging, the iron reduction potential increased positively with increasing hydroethanolic extract and powder concentration. The EOHE exhibited the best activity.




AAE: ascorbic acid equivalents, EOHE: Hydroethanolic extract of *Eribroma oblongum*; EOP: *Eribroma oblongum* powder fraction < 125µm

***Figure 2.*** *Ferric reducing antioxidant power (FRAP) of hydroethanolic extract and powder fraction < 125µm of Eribroma oblongum at different concentrations.*

### *3.3.2. Total Antioxidant Capacity (TAC)*

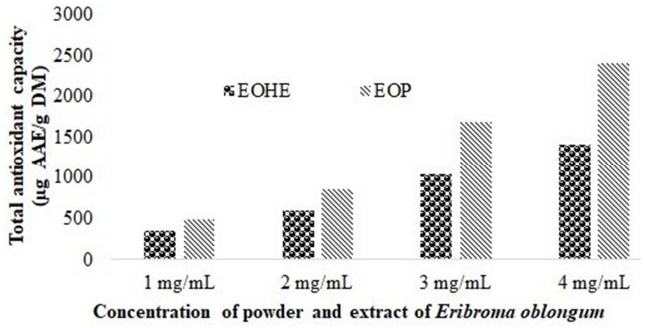



***Figure 3.*** *Total antioxidant capacity (TAC) hydroethanolic extract and powder fraction < 125µm of Eribroma oblongum at different concentrations.*

AAE: Ascorbic Acid Equivalents, EOHE: Hydroethanolic extract of *Eribroma oblongum*; EOP: *Eribroma oblongum* powder fraction < 125µm

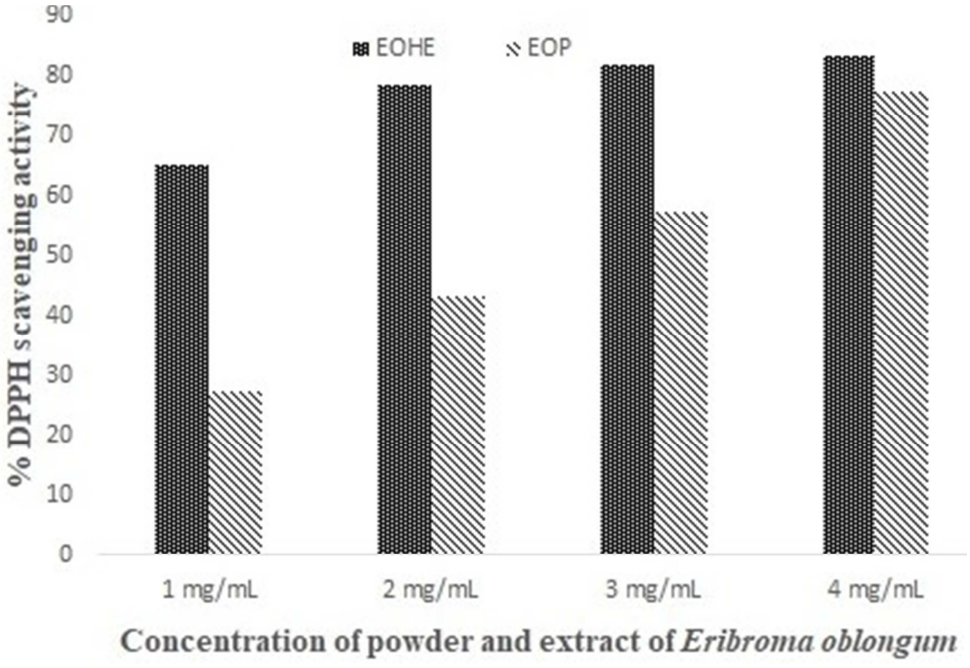
Taking a look at Figure 3 for data on the total antioxidant capacity of the EOP and EOHE, these results confirm previous trends observed through DPPH radical scavenging and iron reduction activities, which showed that the antioxidant potential of our extracts was concentration-dependent. However, the best activity here was shown by the EOP, in contrast to the result observed for FRAP potential.

### *3.3.3. DPPH Radical Scavenging Activity*

Figure 4 shows the DPPH radical scavenging activity of the EOP and EOHE. After observation, it was noted that the different samples trapped this synthetic radical in a concentration-dependent manner (activity increases in parallel with increasing extract or powder concentrations). The EOP with a $CI_{50}$ of 93.14mg/mL exhibited the best activity.

$$Y(_{EOD}) = 35.446 \ln(x) + 3.136; R^2 = 0.9005 \; CI_{50} = 141.80mg/mL$$

$$Y(_{EOP}) = 25.464 \ln(x) - 6.4754; R^2 = 0.7272 \; CI_{50} = 93.14mg/mL$$




EOHE: Hydroethanolic extract of *Eribroma oblongum*; EOP: *Eribroma oblongum* powder fraction < 125µm

***Figure 4.*** *Percentage of DPPH scavenging activity of hydroethanolic extract and powder fraction < 125µm of Eribroma oblongum at different concentrations.*

### *3.4. Influence of Powder and Hydroethanolic Extract of Eribroma oblongum on Interleukin-17 Expression*

According to the results presented in Figure 5, atherogenic feeding of rats without treatment (negative control) resulted in a significant *(P <0.0001*) increase in IL-17 concentration of 35.5% compared with rats in the normal control group.

Treatment with EOP (100 mg/kg bw) in rats fed the atherogenic diet resulted in a significant (*p<0.001*) decrease in IL-17 concentration of 40.98% compared with rats in the negative control group. Whereas treatment with EOHE (100 mg/kg bw) in rats fed the atherogenic diet resulted in a significant (*p<0.001*) increase in IL-17 of 84.58% concentration compared with rats in the negative control group.

The results show that hepatic IL-17 concentration was significantly reduced (*p<0.001*) in rats treated with EOP of 34.66%, compared with rats in the EOHE group.

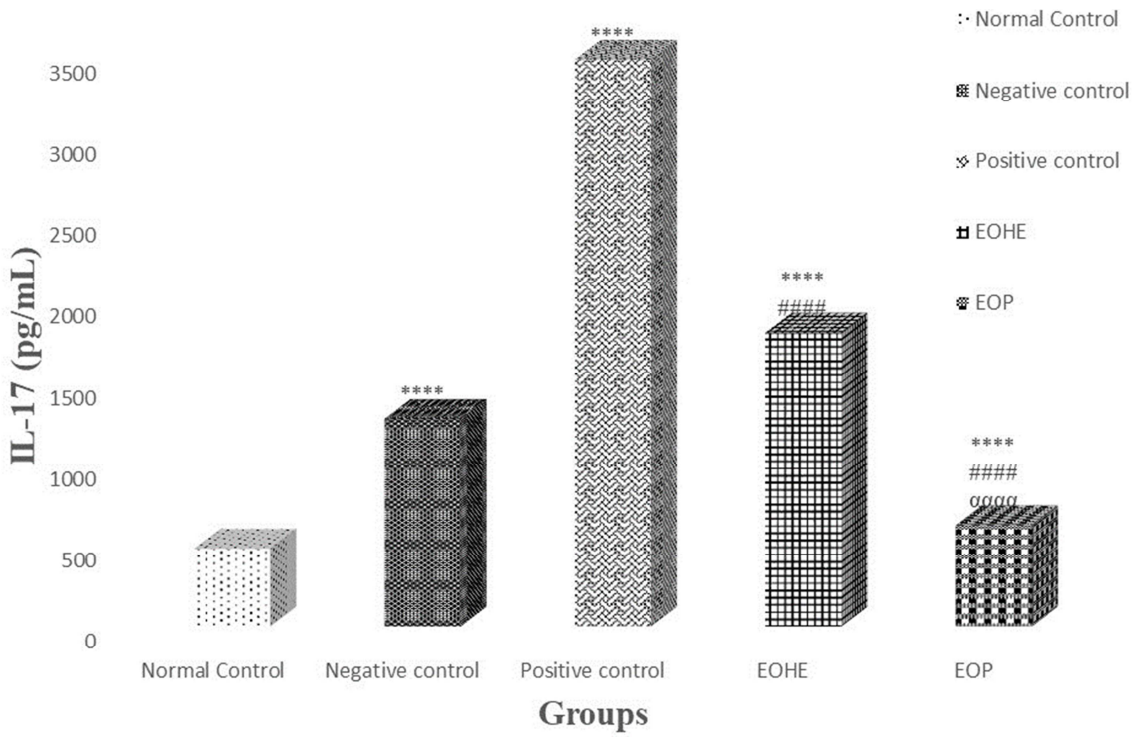



***Figure 5.*** *Influence of hydroethanolic extract and powder fraction < 125µm of Eribroma oblongum on the expression level of Interleukin-17, determined by ELISA assays.*

EOHE: Hydroethanolic extract of *Eribroma oblongum*; EOP: *Eribroma oblongum* powder fraction < 125µm; IL-17: Interleukin-17
Values are expressed as means ± SEM; n=4; $^{****}p<0.00$: significant differences from normal control; $^{####}p<0.00$: significant differences from negative group; $^{\alpha\alpha\alpha\alpha}p<0.001$ significant differences from EOHE treatment group

# 4. Discussion

This work aimed to evaluate some minerals, carry out phytochemical characterization, assess antioxidant potential *in vitro*, and demonstrate the influence of Interleukin-17 on hydroethanolic extract and powder of *E. oblongum* in Wistar rats with steatotic liver induced by feeding an atherogenic diet.

Sieving resulted in a significant increase in the moisture (+52.50%), ash (+85.53%), Iron (+46.40%), Calcium (+81.82%), Manganese (+74.25%), Zinc (+84.20%), and Selenium (+75.67%) content of the < 125 µm fraction compared with the hydroethanolic extract of *Eribroma oblongum*. On the other hand, there was a significant decrease in vitamin C content (-52.26%). This could be explained by the fact that vitamin C is highly sensitive to heat during the grinding of the plant's stem bark [21].

The results show that polyphenol content was significantly high in the EOHE and EOP. Flavonoid content was significantly high in EOP. The justification for such results would initially be attributable to the extraction method, as several works have highlighted the role played by certain physicochemical parameters during extraction and in determining the contents of bioactive compounds [22, 23]. Indeed, extraction is closely dependent on the polarity of the type of solvent or solvent system used, extraction time, pH, temperature, and even extract composition; other factors such as particle size and the existence of interfering substances can also affect extraction efficiency [22, 24].

Furthermore, the presence of a variety of antioxidant compounds (polyphenols, flavonoids) with different polarities and chemical characteristics (having more or less hydroxyl groups), would also be incriminated, as depending on these criteria they may or may not be soluble in a specific type of solvent. However, it has been shown that polar solvents are generally suitable for extracting polyphenols, as in the case of water-ethanol or water-methanol solvent systems. Moreover, ethanol is known to be a good solvent for phenolic compounds (polyphenols and flavonoids) with the advantage of being safe for human consumption, whereas methanol known for its ability to extract low molecular weight phenolic compounds is highly toxic [25]. According to the work of Pérez-Ochoa [26], the quantities and quality of phenolic compounds (polyphenols, flavonoids) vary according to the plant and environmental conditions. It has also been reported that the cutting, peeling, soaking, and other operations carried out on the plant material are also partly responsible for the reduction in antioxidant compounds, including antioxidant potential. The drop in antioxidant activity after steaming can be explained by the application of high temperatures, which, although they deactivate certain enzymes, cause the degradation of various phytochemical compounds, resulting in a significant decline in antioxidant potential [27, 28].

Atherogenic feeding of rats without treatment (negative control) resulted in a significant increase in IL-17 concentration of 35.5% compared with rats in the normal

control group. Treatment with EOP in rats fed the atherogenic diet resulted in a significant decrease in IL-17 concentration of 40.98% compared with rats in the negative control group. Whereas treatment with EOHE in rats fed the atherogenic diet resulted in a significant increase in IL-17 of 84.58% concentration compared with rats in the negative control group.

IL-17 is a pro-inflammatory cytokine that is ubiquitously expressed in liver cells, which explains its involvement in liver damage. Studies have shown that the frequency of IL-17 cells is significantly elevated in liver disease. IL-17 would appear to play a very important role in stimulating liver inflammation by inducing several types of non-parenchymal liver cells to produce pro-inflammatory cytokines [29, 30]. *In vivo* and *in vitro* studies have shown that phenolic compounds found in natural products can reduce inflammation and liver damage due to the administration of a high-fat diet [31, 32]. *E. Oblongum* has demonstrated a high content of polyphenols, good antioxidant potential (DPPH, FRAP, and TAC) *in vitro*, and the presence of mineral elements such as Zinc [33], Copper [34], Selenium [35], Vitamin C [36], Iron [37], and Manganese [38], which confer anti-inflammatory activities and consequently reduce IL-17 expression levels.

The results show that hepatic IL-17 concentration was significantly reduced in rats treated with EOP of 34.66%, compared with rats in the EOHE group. The Controlled Differential Pulverization and Sieving (CDPS) process described by Deli et al. [39], allowed us to demonstrate that this technique concentrated more of the bioactive compounds.

## 5. Conclusion

This study demonstrated that the powder particle size fraction <125 µm and the hydroethanolic extract of *Eribroma oblongum* have a higher composition of minerals such as Iron, Calcium, Manganese, Zinc, Vitamin C, Selenium as well as polyphenols which allows us to support its antioxidant effects. *In vitro*, antioxidant activities on FRAP, DPPH, and TAC increased with the concentration of the hydroethanolic extract and the powder fraction< 125 µm. An animal experiment inducing hepatitis steatosis by an atherogenic diet proved the bioactivity of the < 125 µm fine powder fraction of *E. oblongum* in significantly reducing IL-17 expression. These results demonstrated that *Eribroma oblongum* can be a source of anti-inflammatory agents in the fight against metabolic diseases.

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## Acknowledgments

The authors gratefully acknowledge the cooperation of Professor Njiki Bikoï Jacky for their kind help.

## Conflicts of Interest

The authors declare no conflicts of interest.

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