

Screening of ten wild fruits extracts from Cameroon's flora for their anti-inflammatory and antioxidant potential

Brice Edie Enang II, Sylvain Nsangou Pechangou *, Frederic Nico Njayou, Marie Louise Medoua and Paul Fewou Moundipa

Department of Biochemistry, Laboratory of Pharmacology and Toxicology, Faculty of Science, University of Yaoundé I, 812 Yaoundé, Cameroon.

World Journal of Advanced Research and Reviews, 2023, 18(01), 781–795

Publication history: Received on 02 April 2023; revised on 14 May 2023; accepted on 17 May 2023

Article DOI: <https://doi.org/10.30574/wjarr.2023.18.2.0854>

Abstract

Background: Wild edible fruits are good source of phenolic compounds such as anthocyanins. Those compounds are known for their anti-inflammatory potential. They interact with many mediators of the physiopathological pathway such as NF- κ B which is at a central position in the inflammatory response in macrophages. This study aimed to determine the anti-inflammatory and antioxidant potential of ten wild edible fruits aqueous extracts.

Methods: The antioxidant potential was evaluate through the DPPH, ABTS and OH radical scavenging activity; the ferric reducing agent power and the total antioxidant capacity assessment. Also, the total phenolic and flavonoids, anthocyanins compounds and vitamin C content were assayed. For the anti-inflammatory activity, a primary macrophage culture was used. Inflammatory mediators such as nitric oxide (NO), alkaline phosphatase, NADPH oxidase and 5-lipoxygenase activity was assayed.

Results: *Alchornea cordifolia* and *Pentaclethra macrophylla* extracts showed the greatest antioxidant activity respectively (Antiradical power (AP)=5.82 mol of DPPH/mg of extract and AP=5.05 mol of DPPH/mg of extract for DPPH scavenging assay); (IC₅₀=1.62 μ g/ml and IC₅₀=1.60 μ g/ml for ABTS radical scavenging assay); (IC₅₀=32.67 μ g/ml and IC₅₀=70.01 μ g/ml for OH radical scavenging assay) and (EC₅₀=54.06 μ g/ml and EC₅₀=17.46 μ g/ml for ferric reducing antioxidant power) with a non-significant difference with the reference compound. For the anti-inflammatory activity, firstly it has been observed that *Alchornea cordifolia*'s extract exhibits the greatest potential against NADPH oxidase activity inhibition (IC₅₀=36.20 μ g/ml); alkaline phosphatase activation (EC₅₀=0.27 μ g/ml) with non-significant difference with the reference compound. Secondly, *Pentaclethra macrophylla*'s extract demonstrated a high ability to inhibit NO production (IC₅₀=5.79 μ g/ml) and 5-lipoxygenase (IC₅₀=1.41 μ g/ml).

Conclusion: From the results obtained the assessed wild fruit extracts appears to be good sources for anti-inflammatory and antioxidant bioactive molecules. They could be beneficial for the research of new modulators for the management of inflammatory disorders.

Keywords: Wild fruits; Anti-inflammatory; Antioxidant; Cameroon

1. Introduction

Inflammatory reaction is one of the first defense lines of the body against pathogens. It is part of the native immune system and it's composed of a multitude of circulating cells that constantly maintain the well-being of the body [1]. Those cells (neutrophils, macrophages, dendritic cells...) act either by doing phagocytosis and/or by secreting some

* Corresponding author: Sylvain Nsangou Pechangou

inflammatory mediators (pro- and anti-inflammatory mediators) in normal amount according to the pathogen to which the response is driven against. When the inflammatory process is not well controlled those reactive oxygen species (ROS) will react against the body himself and lead to many disorders such as lipid peroxidation, protein oxidation and even nucleic acid mutations [2]. Also when the immune response against a pathogen is disproportional, activated sentinel cells will produce a high amount of nitric oxide to permit the dilatation of blood vessel, then a pro-inflammatory cytokine (Leukotriene B₄) will act as a chemo-attractant agent to permit other inflammatory cells to reach at the inflammatory site. Other inflammation mediators such as PGE₂, interleukin 6 (IL-6), interleukin 1 β (IL-1 β), tumor necrosis factor-alpha (TNF- α) and interleukin 8 (IL-8) will contribute to the promotion of prolonged inflammation [3, 4]. Also interleukin 17 could be produced in order to activate neutrophils and monocytes to the inflammatory site as describe on the figure 1 [5]. That overproduction of pro-inflammatory mediator can be maintain over the time and conduct to some inflammatory diseases such as inflammatory bowel diseases, rheumatoid arthritis, Alzheimer's disease...[2]. Inflammatory diseases are considered major threats to human health worldwide. One of the consequences is the activation of some key immune cells that will produce pro-inflammatory enzymes. For example activated macrophages can produce NADPH oxidase in order to facilitate the phagocytosis process [6]. That enzyme will be responsible of the production of some reactive oxygen species necessary for the destruction of the antigen. Many approaches have been explored in order to find ways to manage these threats. Plants has been largely explored during recent years for their high secondary metabolites contain and fruits are pointed to contain a high amount of phenolic compounds and could be very beneficial for the managements of those threats [7-9]. Cameroon has a rich diversity of wild fruits whom biological potential remain non-explored [10]. Thus this study was designed to investigate the anti-inflammatory and the antioxidant potential of ten wild fruits extracts from Cameroon's flora.

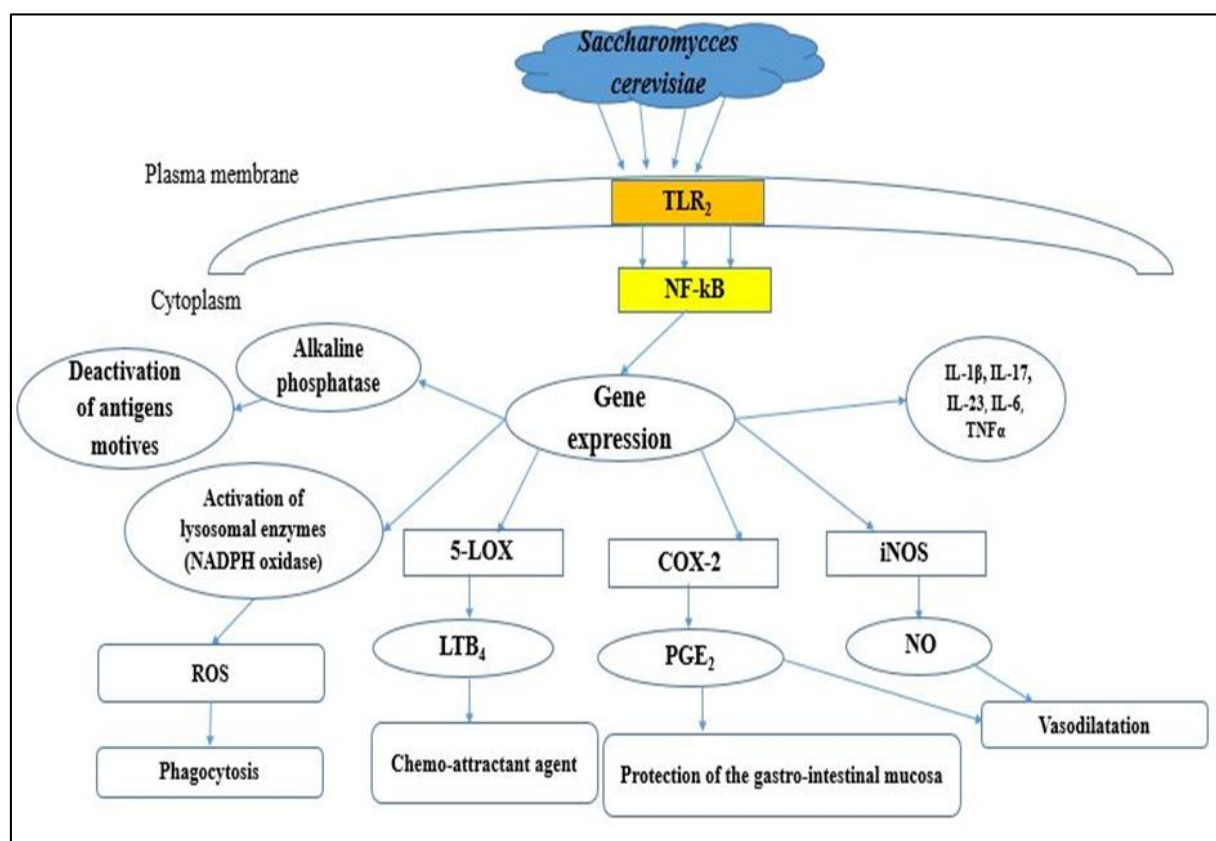


Figure 1 Mechanism of induction of macrophage inflammatory response by *Saccharomyces cerevisiae*

2. Material and methods

2.1. Plant Material

Fresh fruits were collected in 03 region of Cameroon: the Subdivision of Mintom, in the South region, the Subdivision of Fouban, in the West region, and in the subdivision of Mfou, in the Centre region. Harvested specimen were identified at the Cameroon National Herbarium (CNH) in Yaounde, Cameroon. The fruits were washed and rinsed with distilled water and dried in an oven at 50 °C in the laboratory, then crushed in a blender to obtain the powder and conserved.

2.2. Preparation of Extracts

Fifty grams of the powder was boiled in 500µl of distilled water for 30 min and then cooled at room temperature. The resulting mixtures were filtered through Whatman No. 1 paper and dried in an oven at 50 °C. The different fruits extracts were named using a specific nomenclature and different extractions yields were recorded (Table 1).

2.3. Determination of the Antioxidant Potential

The antioxidant potential of the different fruits extracts was evaluated by assessing their 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), and OH radical scavenging activity. Their ferric iron reducing antioxidant power assay, the total antioxidant capacity, phenolic compounds, flavonoids, anthocyanins and vitamin C content was also assessed. For these different tests, extracts at final concentrations of 0.1; 1; 10; 100; and 1000µg/ml were used and tests done in triplicate and results are presented as mean ± standard deviation (SD).

2.3.1. Free Radical Scavenging Activity

The scavenging activity against three radical was assessed (DPPH, ABTS, OH).

DPPH Radical Scavenging Assay

This assay was carried out based on an electron transfer reaction between an antioxidant component and DPPH radical [11]. Briefly in a series of test tubes containing 3.1 ml of the methanolic solution of DPPH (40 µg/ml), fifty µl of plant extracts was added. In the negative and positive control tubes, the extract was replaced respectively by solvent and ascorbic acid. The mixtures were homogenized and incubated in the dark for 30 minutes at room temperature, and the absorbance was measured at 517 nm with a spectrophotometer. The percentage of scavenging was calculated by using the formula (1) above.

$$\% \text{ of scavenging} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{assay}}}{\text{OD}_{\text{control}}} \times 100 \quad (1)$$

Where:

OD_{control} : absorbance of the negative control tube

OD_{assay} : absorbance of the test tube

The 50% inhibitory concentration (IC₅₀) value expressed in µg of extract per mol of DPPH for each extract was determined by using a non-linear regression curve of the DPPH scavenging activity against the logarithm of the concentration of extracts tested. after that the antiradical power (AP) was calculated using the formula (2).

$$\text{AP} = \frac{1}{\text{IC}_{50}} \quad (2)$$

ABTS Radical Scavenging Assay

The stock solution of the ABTS radical was prepared by mixing the ABTS (7 mM) with potassium persulfate (2.45 mM) at room temperature in the dark for 12-16h [12]. The working solution was obtained by calibrating the stock solution to obtain an optical density (OD) of 0.70±0.02 at 734 nm. The ABTS working solution (3.1ml) was mixed with the samples (50 µl) at different concentrations and the absorbance was measured after 10 min of incubation at room temperature at 734 nm using a spectrophotometer. For the negative and positive control tubes, extracts were replaced respectively by water and ascorbic acid. The percentage of scavenging activity was calculated using the formula (1).

The 50% inhibitory concentration (IC₅₀) was calculated the same way as DPPH assay.

OH Radical Scavenging Assay

This assay was performed according to a previously described method [13]. In the test tubes, 0.7ml of FeSO₄ at 3mM, was add to 1ml of H₂O₂ at 1mM. 1.4 ml of distilled water was add to the medium and the volume was completed with 0.4ml of salicylic acid at 10mM. All the mix was stirred then incubated at 37 °C during 1h. The absorbance of the mixture was read at 562nm.

2.3.2. Ferric Reducing Antioxidant Power Assay

The ferric reduction power assay was performed following the Fenton reaction [14]. In each tube 50 µl of plant extracts, 1100 µl of phosphate buffer (0.6 M pH 6.6), 1000 µl of 0.25% potassium ferricyanide were added. In the blank, 1100 µl of distilled water was added instead of potassium ferricyanide. After incubation for 20 min at 50 °C, 1 ml of 10% trichloroacetic acid (TCA) was added to all tubes. Tubes were centrifuged (1620g, 10 min 4 °C) then, to 1ml of supernatant was added 1ml of distilled water and 200 µl of ferric chloride. The whole was well homogenized and then left to stand for 10 min. The absorbance was measured at 700 nm against the blank in a spectrophotometer and the percentages of iron reduction were calculated using the formula (1) and EC₅₀ was also determined as previously described.

2.3.3. Determination of the Total Antioxidant Capacity (TAC)

The TAC was measured following the method previously described [15]. In each test tube 50 µl of plant extracts, 1ml of 0.6 M sulfuric acid, 1050 µl of 28 mM sodium phosphate and 1050 µl of 4 mM ammonium molybdate were successively added. The tubes were capped with the beads and heated for 90 min then cooled on a stream of cold water. The absorbance of the blue staining mixture was measured in a spectrophotometer at 695 nm. The antioxidant capacity of the extracts expressed in milligram equivalent ascorbic acid per g of extract (mg Eq AA/g of extract) was determined from the calibration curve obtained by using ascorbic acid.

2.3.4. Determination of Total Phenolic Compounds Content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [16]. In each tube, we added 2000 µl of distilled water, 100 µl of extract at 100 µg/ml and 200 µl of Folin-ciocalteu (2N) solution. After 3 min of incubation at room temperature, 1000 µl of 20% Sodium Carbonate was added. The mixture was incubated for 1 hour in the dark at room temperature, and absorbance read at 700 nm. The total phenolic compounds contents were expressed in milligram equivalents of gallic acid per gram of extract (mg GAE/g extract) using a calibration curve.

2.3.5. Determination of Flavonoids Content

The total flavonoids content was determined by the aluminum chloride complexation method [16]. In the blank and test tubes were respectively added, 500 µl of methanol and 500 µl of the extract solution then, 500 µl of aluminum chloride (AlCl₃) 2% (w/v) was introduced in each of the tubes. All tubes were vortexed and incubated at room temperature for 1 hour and the optical densities of the yellow stain read at 430 nm against the blank. The total flavonoids contents were expressed in milligram equivalents of Quercetine per gram of extract (mg Eq Q/g extract) using a calibration curve.

2.3.6. Determination of Anthocyanins Content

The total monomeric anthocyanins concentration was determined by the pH differential method based on the structural transformation that occurs with a change in pH (colored at pH 1.0 and colorless at pH 4.5). [17]. 0.5ml of fruits extracts were added to 2ml of pH 1.0 buffer solution (potassium chloride, 0.025M) on one hand. On the other hand 0.5ml of extract was added to 2ml of pH 4.5 buffer solution (sodium acetate, 0.4M). The medium was left at room temperature and the absorbance were read both at 520nm and 700nm within 20-50 min of preparation. Anthocyanins concentration was calculated and expressed as cyanidin-3-glucoside equivalents using the above formula.

$$[\text{Anthocyanins}] = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times l \times \alpha}$$

Where

A= (A_{520nm} - A_{700nm})pH 1.0 - (A_{520nm} - A_{700nm})pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyaniding-3-glucoside (cyd-3-glu);

DF= dilution factor; l= path length in cm;

ε= 26900 molar extinction coefficient in L.mol⁻¹.cm⁻¹ for cyd-3-glu;

α= quantity of extract in the tube (in g);

[Anthocyanins] = anthocyanins content in µg/g of plant extract.

2.3.7. Determination of Ascorbic Acid Content

The determination of total vitamin C content was conducted by a spectrophotometric method using potassium permanganate as a chromogenic reagent. The absorbance was measured when a potassium permanganate solution

reacted with the solution of ascorbic acid in acid medium. Vitamin C consumes KMnO_4 (purple color) causing a decrease in the absorbance at 525-530nm [18]. The vitamin C contents was expressed in milligram per gram of extract (mg/g extract) using a calibration curve.

2.4. Evaluation of the Anti-inflammatory Activity

2.4.1. Cell Line and Culture

Primary macrophages was isolated from mouse. For the elicitation, an intra-peritoneal injection of 0.5 ml of a 2% starch solution (inflammatory agent) was done [19]. Four days after, the animal was sacrificed by cervical dislocation. Subsequently, 5 ml of PBS buffer (0.1 M, pH 7.4) were injected into the mice peritoneal cavity with a syringe. After massaging the abdominal cavity of the animal, the injected buffer was then slowly aspirated having done a small incision through the abdomen. The resulting solution containing macrophages was put into 15 ml Falcon tubes and kept in ice. The resulting fluid was centrifuged (1620g, 4 °C, 10 min) and the supernatant removed. Erythrocytes were removed by osmotic shock [16] by suspending the cells in 1 ml of hypotonic 0.05 M NaCl solution for 1 min. The isotonicity was then restored by adding 1 ml of 0.25 M NaCl. The mixture was centrifuged again (1620 g, 4 °C, 10 min), and the resulting pellet containing mostly macrophages was suspended in 2 ml of DMEM culture medium (containing 0.2% streptomycin and penicillin, 0.4 g/l of bovine serum albumin, and 3.7g/l NaHCO_3 in PBS and filtered with 0.22 μm Millipore filters) and kept in ice.

The cell viability was determined by using the trypan blue exclusion method [20].

2.4.2. Treatment of Isolated Macrophages with Tested Samples

On the 96-well plate, 150 μl of a cell suspension (10^4 cells/well) were distributed in different wells. In the test and positive control wells, 50 μl of *Saccharomyces cerevisiae* (SC) (250 $\mu\text{g/ml}$) were added to stimulate macrophage to produce pro-inflammatory cytokines; 50 μl of DMEM were added in the blank well. The micro-plate was incubated for 1h at 37 °C (5% CO_2), then 50 μl of plant extracts or baicalin at different concentrations (0.1, 1, 10 and 100 $\mu\text{g/ml}$) were added to the test wells and 50 μl of DMEM were added to the blank and positive control wells. After 3h of incubation at 37 °C (5% CO_2), the cells supernatants were used for the nitric oxide quantification while the pellets were used for the activity of lysosomal enzymes, 5-lipoxygenase and cytotoxicity.

2.4.3. Cell Viability Assay

The effect of fruits extracts on the primary culture macrophage viability was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) as described elsewhere [21]. The cell pellet from the different incubations was taken up in 100 μl of MTT solution (0.5 mg/ml in PBS) and the mixture was incubated at 37 °C for 1h 30 min, then the supernatant was removed and 100 μl of acidified isopropanol was added to each tube to dissolve the formazan crystals formed. Finally, the absorbance of the purple solution was read at 630 nm against the acidified isopropanol solution. The percentages of cell viability were calculated using the following formula:

$$\% \text{ viability} = (\text{OD}_{\text{assay}} / \text{OD}_{\text{control}}) \times 100$$

2.4.4. Quantification of Nitric Oxide Production by Stimulated Macrophages

The assay was done according to the Griess diazotation method already described [22]. One hundred microliter of cell supernatants obtained previously were mixed with 100 μl of Griess reagent (1% sulphonylamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% v/v phosphoric acid). The mixture was incubated at room temperature for 10 min and the absorbance was measured in a plate reader at 550 nm. The amount of nitrite is measured against the standard sodium nitrate curve.

The percentage inhibition of nitric oxide production was calculated according to the formula

$$\% \text{ inhibition} = [(\text{OD}_{\text{control}} - \text{OD}_{\text{assay}}) / \text{OD}_{\text{control}}] \times 100$$

2.4.5. Measurement of Inflammatory Enzymes Activities

Alkaline Phosphatase Activity

The measurement of this enzyme activity was done as described [23]. The cell pellets obtained in the methodology were used. The pellets obtained were solubilized with 25 μl of Triton X-100. Then, 50 μl of p-nitrophenylphosphate (10 mM)

and 50 µl of glycine buffer (0.1 M, pH 9.0) was added to the medium. All solutions were incubated for 30 min at 37 °C. The reaction was stopped by adding 100 µl of NaOH buffer (0.2 M, pH 12) and the absorbance was measured at 405 nm.

The percentage of variation of the enzyme activity was calculated taking into account the control tubes using to the following formula:

$$\% \text{ of activity} = [(OD_{\text{assay}} - OD_{\text{control}}) / OD_{\text{control}}] \times 100$$

NADPH Oxidase Activity

Micro-plates were used for this experiment and the protocol used was the one previously described [6]. Here 150µl of cells (10⁴cells/ml) were incubated with 50µl of fruits extracts at 37 °C, 5% CO₂ during 3h. After that, 50µl of *Saccharomyces cerevisiae* and 50µl of nitro blue tétrazolium (NBT) was added to the medium then incubated at 37 °C, 5% CO₂ during 1h. The supernatant was eliminate, the pellets was solubilized inside 20µl methanol then left at room temperature during 10min. After that 50µl of KOH 2M and 50 of DMSO was added to the medium. The absorbance was read at 570nm.

5-Lipoxygenase Activity

This test was performed according to a method previously described [24]. Primary macrophages were isolated and recovered into DMEM as already described [16]. A cells volume of 950µl was putted inside each tube (10⁵ cells/tube). Then 300µl of *Saccharomyces cerevisiae* suspension (250µg/ml) was added. In the negative control tube the antigen was replaced by the culture medium. The mix was then incubated during one hour at 37 °C (5% CO₂). Subsequently 50µl of extract was added into the test tubes and 50µl of medium for the controls, followed by a second incubation of 3 hours. After this each tube was centrifuged at 720g, 10 minutes, 4 °C and the supernatant discarded. The pellet containing the cells was lysate by the addition of 50µl Triton X-100 and the tubes were shaken for 2 minutes. At the end, 1000µl of linoleic acid (125µM) was added and incubated for 30 minutes. Optical densities of the supernatant were read at 234nm.

2.5. Data Analysis

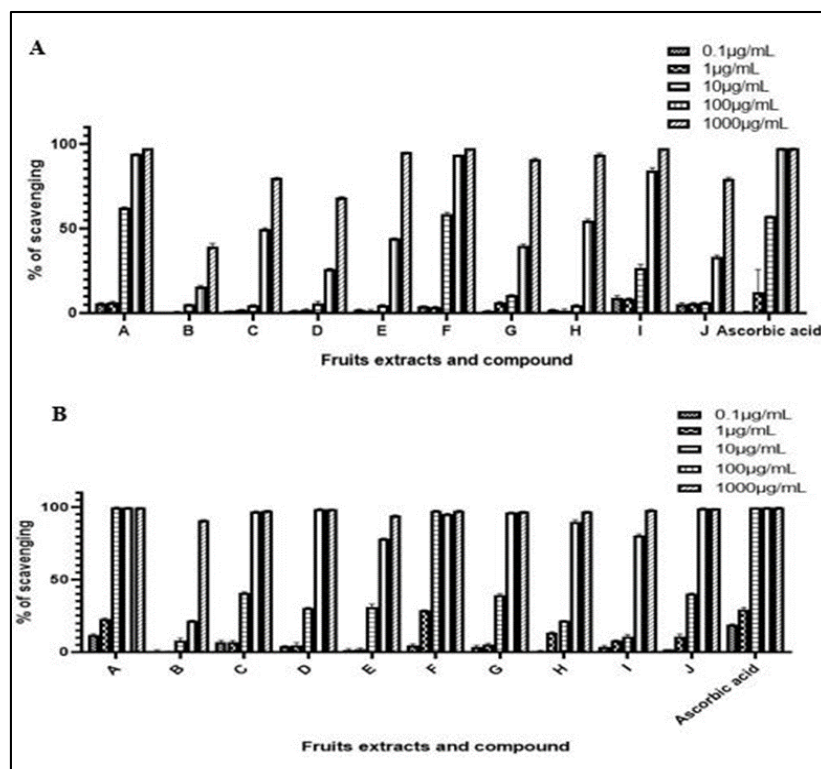
Data analyses were performed using GraphPad Prism 8.0.1 software. The results were expressed as mean ± standard deviation and the sample comparison was done using one-way ANOVA followed by the multiple comparison test of Turkey with a p-value p<0.05.

3. Results

3.1. Antioxidant Activities of the Studied Fruit Extracts

3.1.1. Free Radical Scavenging Activity

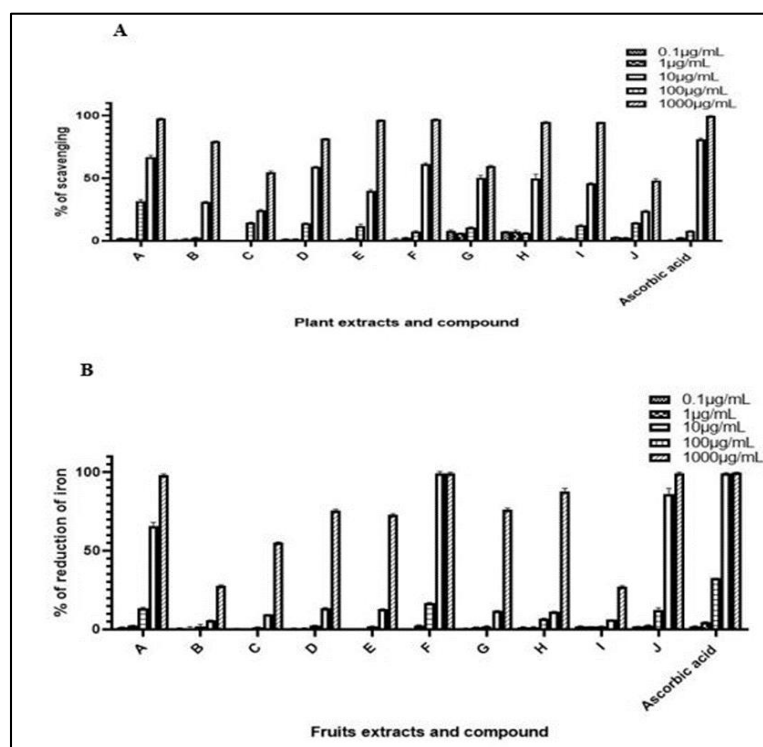
From all the fruit extract tested, *Alchornea cordifolia* and *Pentaclethra macrophylla* revealed the best ability to scavenge free radicals. AP and IC₅₀ are good indicators for that. For DPPH assay, AP were respectively 5.82 ± 1.15, 5.05 ± 0.85 mol of DPPH/mg of extract for the two fruits, with no significant difference with the standard (5.53 ± 1.52 mol of DPPH/mg of AA). For ABTS assay, IC₅₀ were respectively 6.86±0.94, 7.90±0.92 µg/ml, with no significant difference with the standard (7.23±1.35 µg/ml). For OH assay, IC₅₀ were respectively IC₅₀=32.67±2.56, 70.01 ± 1.99 µg/ml (Table 1).



A= *Alchornea cordifolia*, B= *Aframomum melegueta*, C= *Aframomum melegueta* peelings, D= *Ficus benjamina*, E= *Landolphia hispida*, F= *Pentaclethra macrophylla*, G= *Musanga cecrepioides*, H= *Panda oleosa*, I= *Pseudospondias microcarpa*, J= *Uapaca staudtii*

Figure 2 DPPH and ABTS radical scavenging activity. A: DPPH radical scavenging, B: ABTS radical scavenging

3.1.2. Iron Reducing Power (FRAP) of Fruits Extracts



A= *Alchornea cordifolia*, B= *Aframomum melegueta*, C= *Aframomum melegueta* peelings, D= *Ficus benjamina*, E= *Landolphia hispida*, F= *Pentaclethra macrophylla*, G= *Musanga cecrepioides*, H= *Panda oleosa*, I= *Pseudospondias microcarpa*, J= *Uapaca staudtii*

Figure 3 OH radical scavenging and Ferric reducing antioxidant power activity. A: OH radical scavenging activity, B: Ferric reducing antioxidant power activity

The ferric reducing antioxidant power assay revealed that *Pentaclethra macrophylla*'s aqueous extract exhibited the highest reducing activity with an evolution going in a concentration-dependent manner (Fig. 3b). The EC₅₀ obtain (17.46±3.89µg/ml) was no significantly different from that of the reference compound (14.10±3.52µg/ml) (Table 1).

Table 1 IC₅₀ and EC₅₀ for antioxidant activities

Fruits extracts / compound	AP for DPPH radical scavenging	IC ₅₀ for ABTS radical scavenging	IC ₅₀ for OH radical scavenging	IC ₅₀ for ferric reducing antioxidant power
A	5.82 ± 1.15 ^b	1.622 ± 0.81 ^b	32.67 ± 2.56 ^b	54.06 ± 1.97 ^a
B	0.02 ± 0.00 ^a	226.4 ± 6.03 ^a	236.1 ± 3.66 ^a	>1000 ^a
C	0.31 ± 0.01 ^a	12.64 ± 1.41 ^a	720.9 ± 13.54 ^a	814.8 ± 3.97 ^a
D	0.10 ± 0.00 ^a	14.95 ± 1.55 ^a	79.4 ± 4.17 ^a	417.7 ± 4.53 ^a
E	0.33 ± 0.00 ^a	24.75 ± 1.65 ^a	128 ± 4.86 ^a	454.2 ± 3.57 ^a
F	5.05 ± 0.85 ^b	1.609 ± 0.51 ^b	70.01 ± 1.99 ^a	17.46 ± 1.57 ^b
G	0.29 ± 0.01 ^a	13.34 ± 1.20 ^a	255.1 ± 13.51 ^a	431.6 ± 4.57 ^a
H	0.45 ± 0.01 ^a	23.47 ± 2.52 ^a	99.67 ± 4.63 ^a	325.2 ± 7.37 ^a
I	1.73 ± 0.25 ^a	39.1 ± 2.73 ^a	105.5 ± 3.98 ^a	>1000 ^a
J	0.18 ± 0.00 ^a	12.47 ± 1.47 ^a	>1000 ^a	33.24 ± 1.82 ^b
Ascorbic acid	5.53 ± 1.52	1.605 ± 0.74	42.14 ± 1.55	14.10 ± 1.29

a: significantly different to the standard at p<0.05; b: non-significantly different to the standard at p<0.05. AP= antiradical power in mol of DPPH/mg of extract; A= *Alchornea cordifolia*, B= *Aframomum melegueta*, C= *Aframomum melegueta* peelings, D= *Ficus benjamina*, E= *Landolphia hispida*, F= *Pentaclethra macrophylla*, G= *Musanga cecropioides*, H= *Panda oleosa*, I= *Pseudospondias microcarpa*, J= *Uapaca staudtii*

3.1.3. Total Antioxidant Capacity of Fruits Extracts

The determination of the total antioxidant capacity was done by the phosphomolybdenum method. The results expressed in milligram ascorbic acid equivalent per gram of plant extract (mgEAA/g extract) show us that *Alchornea cordifolia*'s extracts has the greatest ability to reduce the phosphomolybdc complex (213.92 ± 1.03 mgEq AA/g extract) (Table 2).

3.1.4. Total Phenolic Compounds and Flavonoids Content of Fruits Extracts

The determination of phenolic compounds with Folin Ciocalteu reagent showed that *Pentaclethra macrophylla*'s extract contained the greatest amount of phenolic compounds compare to the other extracts (42.86 ± 0.08 mg/g of extract). In addition, the determination of flavonoids by the aluminium chloride complexation method showed that the aqueous extract of *Alchornea cordifolia* contained more flavonoids than the others (5.62 ± 0.22 mg/g of extract) (Table 2).

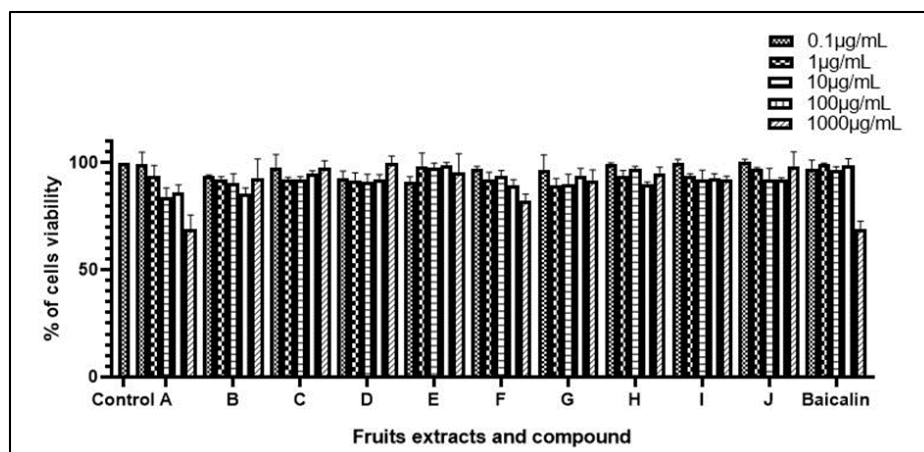
3.1.5. Total Monomeric Anthocyanins and Vitamin C Content of Fruits Extracts

The pH differential method reveal that the anthocyanins content was ranged from 8.35 to 243.80 µg/g of plant extract. *Ficus benjamina* and *Pentaclethra macrophylla*'s extract have the greatest anthocyanins content (Respectively 243.80 and 195.38µg/g of plant extract). The vitamin C content assessed were ranged from 2.07 mg/g of extract for *Pseudospondias microcarpa* to 38.86 ± 0.99 mg/g of extract for *Ficus benjamina* fruits extract (38.86 ± 0.99 mg/g of extract). (Table 2).

3.2. Anti-Inflammatory Activities of the Fruit Extracts

3.2.1. Cell Cytotoxicity of Fruits Extracts Using MTT Assay

The evaluation of the cytotoxicity of those plant extracts on primary macrophages culture was carried out using MTT assay at extract concentrations ranged from 0.1 to 1000µg/ml (Fig. 4). The results obtained show that below 1000µg/ml, fruit extract are non-toxic for macrophages in culture.

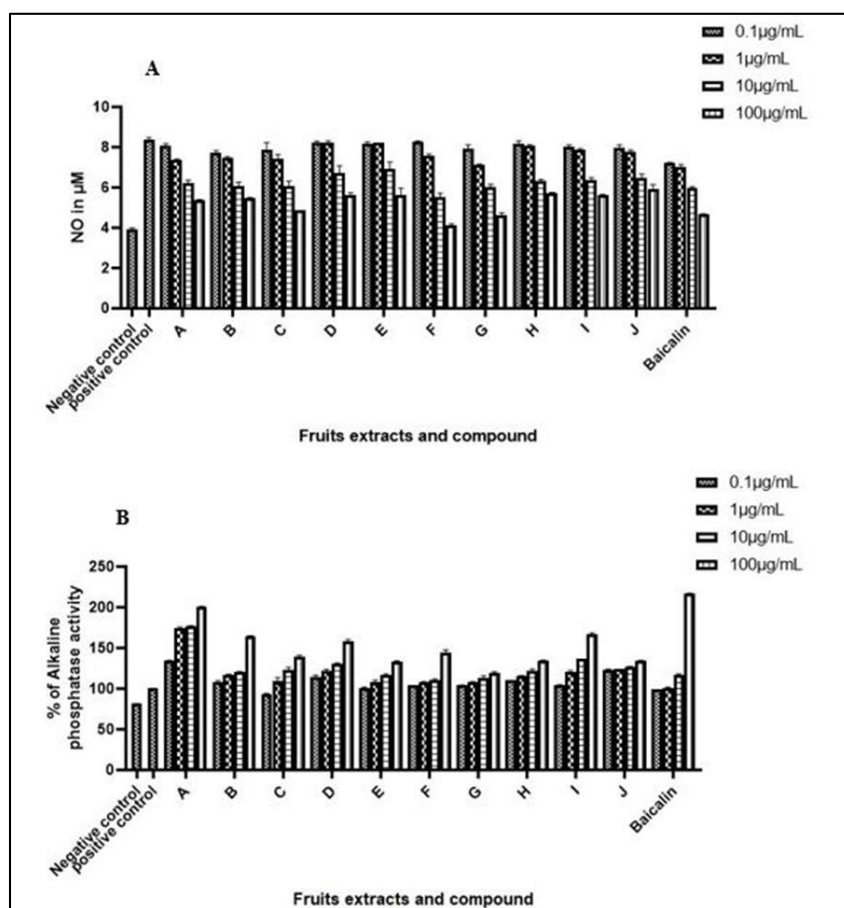


A= *Alchornea cordifolia*, B= *Aframomum melegueta*, C= *Aframomum melegueta* peelings, D= *Ficus benjamina*, E= *Landolphia hispida*, F= *Pentaclethra macrophylla*, G= *Musanga cecrepioides*, H= *Panda oleosa*, I= *Pseudospondias microcarpa*, J= *Uapaca staudtii*

Figure 4 MTT viability assay

3.2.2. Inhibitory Effect of Fruits Extracts on Nitric Oxide Production

The ability of the fruit extracts to inhibit nitric oxide synthesis by SC-activated macrophages was carried out using the Griess diazotization method. It was found that the plant extracts significantly ($p < 0.05$) reduced NO production by macrophages (Fig. 5a). *Pentaclethra macrophylla*'s extract showed a pronounced inhibitory activity ($5.79 \pm 0.79 \mu\text{g/ml}$) with no significant difference to that of the reference compound ($5.08 \pm 1.65 \mu\text{g/ml}$). (Table 3).

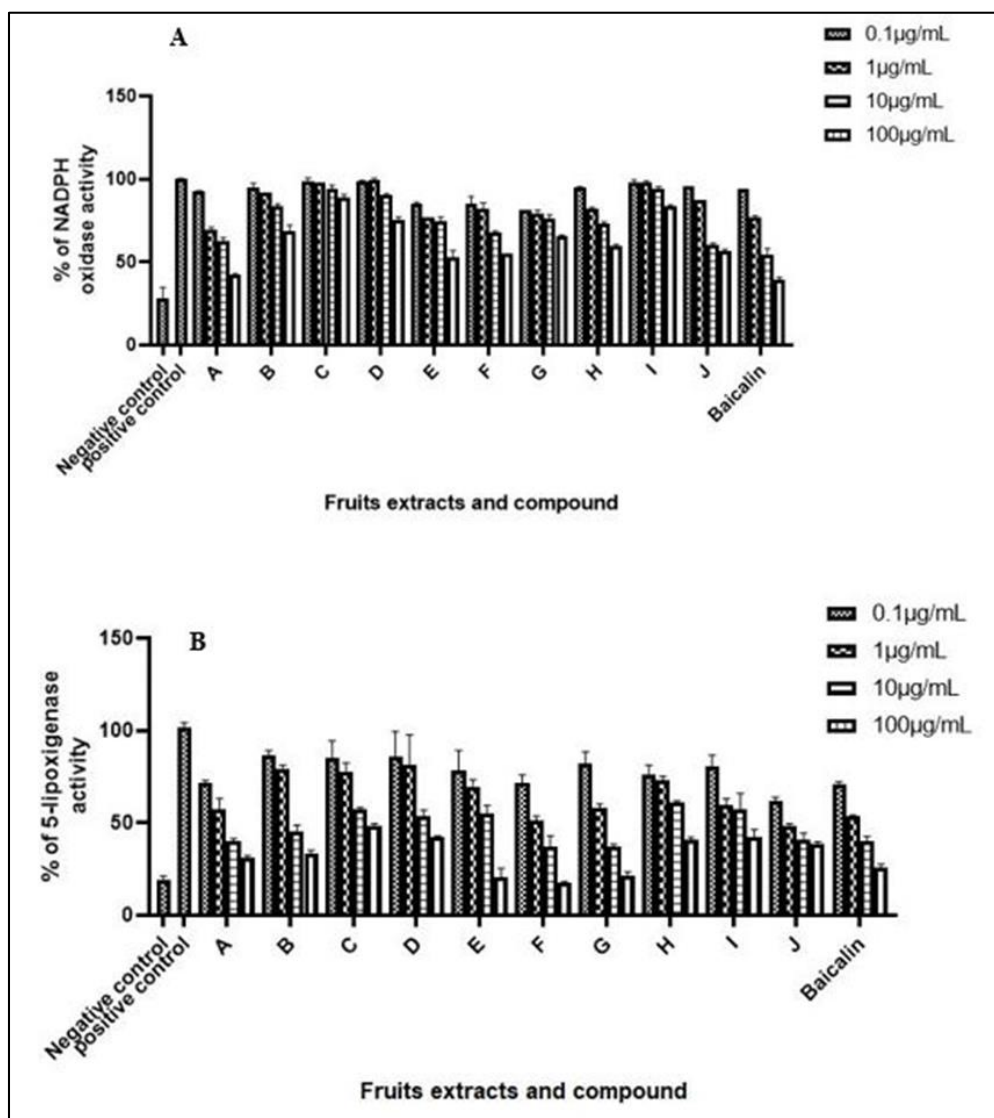


A= *Alchornea cordifolia*, B= *Aframomum melegueta*, C= *Aframomum melegueta* peelings, D= *Ficus benjamina*, E= *Landolphia hispida*, F= *Pentaclethra macrophylla*, G= *Musanga cecrepioides*, H= *Panda oleosa*, I= *Pseudospondias microcarpa*, J= *Uapaca staudtii*

Figure 5 Modulating effect of fruits extracts on nitric oxide production and alkaline phosphatase activity. A: Inhibitory effect against nitric oxide synthesis, B: Stimulating effect against alkaline phosphatase activity

3.2.3. Modulating Effect of Fruits Extracts on Some Key Inflammatory Enzymes

Firstly the activity of an anti-inflammatory lysosomal enzyme (alkaline phosphatase) was measured and it was observed that it was considerably increased in the presence of those fruits extracts (Fig. 5b). The calculation of the EC_{50} allowed us to show that the *Alchornea cordifolia*'s extract boosts alkaline phosphatase activity better than baicalin which was our reference anti-inflammatory compound (Respectively $0.27 \pm 0.16 \mu\text{g/ml}$ and $12.47 \pm 1.32 \mu\text{g/ml}$). Secondly the inhibitory effect on a pro-inflammatory enzyme (NADPH oxidase) was measured. IC_{50} 's obtained show that *Alchornea cordifolia*'s aqueous extract has a great inhibitory activity with no significant difference with the standard ($p < 0.05$). Finally, the inhibitory effect on another pro-inflammatory enzyme (5-lipoxygenase) was measured. This inhibition was more pronounced in the presence of the *Pentaclethra macrophylla*'s extract with a non-significant difference with the reference compound (Table 3).



A= *Alchornea cordifolia*, B= *Aframomum melegueta*, C= *Aframomum melegueta* peelings, D= *Ficus benjamina*, E= *Landolphia hispida*, F= *Pentaclethra macrophylla*, G= *Musanga cecrepioides*, H= *Panda oleosa*, I= *Pseudospondias microcarpa*, J= *Uapaca staudtii*

Figure 6 Inhibitory effect against some key pro-inflammatory enzymes activities. A: Inhibitory effect against NADPH oxidase activity, B: Inhibitory effect against 5-lipoxygenase activity

Table 3 IC₅₀ and EC₅₀ for anti-inflammatory activities

Fruits extracts / compound	IC ₅₀ for NO inhibition	EC ₅₀ for Alkaline phosphatase activation	IC ₅₀ for NADPH oxidase inhibition	IC ₅₀ for 5-lipoxygenase inhibition
A	17.33 ± 2.20 ^a	0.27 ± 0.16 ^b	36.20 ± 4.90 ^b	3.04 ± 1.08 ^b
B	17.02 ± 2.91 ^a	47.57 ± 5.33 ^b	>500 ^a	12.50 ± 2.47 ^a
C	10.19 ± 3.47 ^b	214.10 ± 16.43 ^b	>500 ^a	60.14 ± 8.47 ^a
D	37.48 ± 4.48 ^a	53.53 ± 5.69 ^b	>500 ^a	29.75 ± 7.74 ^a
E	42.94 ± 4.75 ^a	>500 ^b	259.90 ± 22.31 ^b	7.84 ± 2.52 ^b
F	5.799 ± 0.79 ^b	144.1 ± 8.12 ^b	224.50 ± 14.11 ^b	1.41 ± 0.74 ^b
G	7.34 ± 1.31 ^b	>500 ^a	>500 ^a	2.83 ± 0.94 ^b
H	33.87 ± 5.01 ^a	>500 ^a	342.9 ± 13.83 ^b	34.75 ± 5.60 ^a
I	30.53 ± 3.80 ^a	25.12 ± 2.38 ^b	>500 ^a	22.64 ± 6.56 ^a
J	49.71 ± 5.95 ^a	>500 ^a	129.6 ± 15.27 ^b	1.54 ± 1.08 ^b
Baicalin	5.09 ± 1.65	12.47 ± 1.32	24.29 ± 3.14	2.09 ± 0.66

a: significantly different to the standard at p<0.05; b: non-significantly different to the standard at p<0.05.

A= *Alchornea cordifolia*, B= *Aframomum melegueta*, C= *Aframomum melegueta* peelings, D= *Ficus benjamina*, E= *Landolphia hispida*, F= *Pentaclethra macrophylla*, G= *Musanga cecrepioides*, H= *Panda oleosa*, I= *Pseudospondias microcarpa*, J= *Uapaca staudtii*

4. Discussion

Amongst all the plants selected in this study, *Alchornea cordifolia* and *Pentaclethra macrophylla* had the best antioxidant and anti-inflammatory activity. Generally, the antioxidant capacity of a biological material need to be evaluated by using more than one method in order to ensure the reliability of the different conclusion that will be taken at the end of the work [26]. The antioxidant potential of those ten wild fruits extracts were evaluated in this work using different methods (DPPH, ABTS and OH radical scavenging assays, ferric reducing antioxidant power assay and total antioxidant capacity determination). Firstly they were able to act against oxidative stress through the scavenging of some active radicals, such as the scavenging of OH radical that could limit some of its deleterious effects such as lipid membrane peroxidation and protein oxidation. Also DPPH and ABTS radical scavenging potential of the extracts demonstrate their ability to transfer and electron in order to stabilize those radicals [26,27] Moreover their ability to reduce ferric iron to its ferric state stands as a greater effect. In fact the ferrous iron is highly involved in the Fenton reaction that lead to the production of reactive oxygen species. Those results are similar to the one obtain with *Codiaeum variegatum* ethanolic extract that was able to inhibit lipid membrane peroxidation.[25, 28] This antioxidant and antiradical activity observed could be due to the presence of phenolic compounds contained in those extracts. Knowing that the antioxidant potential of those secondary metabolites is well establish, those activities could be correlated to their amount in those fruits extracts [25, 29]. At the end of this first part, assessed wild fruit extracts can be classify from the one having the highest antioxidant potential to the one having the lowest as follow: *Alchornea cordifolia* > *Pentaclethra macrophylla* > *Aframomum melegueta* peelings > *Panda oleosa* > *Landolphia hispida* > *Ficus benjamina* > *Musanga cecrepioides* > *Pseudospondias microcarpa* > *Uapaca staudtii* > *Aframomum melegueta*.

The inflammatory response is one of the first reaction of the human body to and aggression [30]. Many cells are involved, amongst whom we have macrophages [31,32]. They are one of the most studied inflammatory cells due to their high implication in that inflammatory process. In order to mimic what exactly happens in the human body a primary culture of intra-peritoneal macrophage was used [20]. In presence of an antigen such as *Saccharomyces cerevisiae*, its antigen motives will be recognized by Toll-Like Receptors 2 (TLR-2) and will lead to the synthesis of many pro-inflammatory mediators [33]. First of all that recognition will conduct to the translocation of the nuclear factor NF-κB inside the nucleus where it will act at many levels [33, 34]. That factor will activate the expression of inducible nitric oxide synthase and lead to a synthesis of a high amount of a vasodilative agent (nitric oxide) [35]. Alkaline phosphatase will be produce in order to repress the activation of NF-κB and to inactivate by dephosphorylation some antigen motives (LPS, flagellin...) [36]. Also NADPH oxidase will be synthesized (Fig. 1). This lysosomale enzyme involve in phagocytosis is responsible for the synthesis of reactive oxygen species that will contribute to the destruction of the pathogen. At the

same time phospholipase 2 will be activate and will produce the substrate for 5-Lipoxygenase. It's this last enzyme that will produce a chemo-attractant agent (Leucotrien B4) that will permit attract other immune cells at the inflammatory site [37].

All the fruits extract used in this work was at least non-toxic for macrophages cell in culture at a concentration below 1000µg/ml as observed with the MTT cells cytotoxicity assay. The anti-inflammatory activity of those extracts was then evaluated at a maximum concentration of 100µg/ml. Firstly their ability to inhibit the synthesis of nitric oxide revealed that *Pentaclethra macrophylla* was more effective ($5.799 \pm 0.79 \mu\text{g/ml}$) with non-significant difference with the reference compound. NO and superoxide anion are highly synthesized when macrophages are stimulated by a specific antigen. An excessive accumulation of those two agents may induce the formation of peroxynitrite (OONO-), and cause many inflammatory disorders. The inhibition of any molecular target involved in the NO pathway of inflammation such as ROS, iNOS, and NO itself could have potential to inhibit inflammatory responses. This prove its potential to limit dilatation due to this pro-inflammatory mediator [38, 39]. *Alchornea cordifolia* had the greatest capacity to activate alkaline phosphatase up to 200.80% of its normal activity at 100µg/ml. That could be necessary to limit some pro-inflammatory mediators synthesis through the dephosphorylation of antigen motives [36]. NADPH oxidase activity was highly reduced by *Alchornea cordifolia*'s extracts up to 42.05% of the initial activity at 100µg/ml. This result suggest that this fruit extract is able of to modulate the phagocytosis through the reducing of reactive oxygen species synthesis by NADPH oxidase (Nox-2) [40]. *Pentaclethra macrophylla*'s extract has inhibit more effectively the activity of 5-lipoxygenase up to 17.43 % of the normal activity at 100µg/ml. This shows its ability to prevent an uncontrolled recruitment of immune cells at the inflammatory site due to the inhibition of the synthesis of the chemo-attractant mediator responsible for that [37, 41]. Moreover the anthocyanins level observed could be a great marker for the potential of those extract to treat and prevent inflammatory related diseases such as inflammatory bowel disease. Because it has already been demonstrated that anthocyanins can act at many level of the physiopathology of these disease in order to contribute to its management [42]. At the end of this second part, assessed wild fruit extracts can be classify from the from the one having the highest anti-inflammatory potential to the one having the lowest as follow: *Pentaclethra macrophylla* > *Alchornea cordifolia* > *Aframomum melegueta* > *Panda oleosa* > *Musanga cecrepioides* > *Uapaca staudtii* > *Landolphia hispida* > *Ficus benjamina* > *Aframomum melegueta* peelings > *Pseudospondias microcarpa*.

Abbreviations:

- **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
- **COX-2:** Cyclo-oxygenase-2
- **DMEM:** Dulbecco's Modified Eagle Medium
- **DPPH:** 2,2-diphenyl-1-picryl hydrazyl
- **EC₅₀:** Efficiency concentration 50
- **FRAP:** Ferric reducing antioxidant power
- **IC₅₀:** Inhibitory concentration 50
- **IL:** Interleukine
- **iNOS:** inducible nitric oxide synthase
- **LTB₄:** Leucotrien B4
- **MTT:** 3(4,5-dimethylthiazol-2-yl)-2,3diphenyletrazolium
- **NADPH:** reduced nicotinamide adenine dinucleotide phosphate
- **NF-κB:** Nuclear Factor-kappa B
- **NO:** Nitric Oxide
- **PGE₂:** Prostaglandine E2
- **RNOs:** reactive nitrogen oxide species
- **SC:** *Saccharomyces cerevisiae*
- **TLR:** Toll-liked Receptor
- **TNF-α:** Tumor Necrosis Factor alpha
- **5-LOX:** 5-lipoxygenase

5. Conclusion

All the results obtained in this work effectively confirm the anti-inflammatory potential of those different fruits extracts through the inhibition of some key pro-inflammatory mediators and enzymes (nitric oxide, NADPH oxidase, and 5-Lipoxygenase); and the activation of an anti-inflammatory enzyme (alkaline phosphatase). Also the antioxidant potential was proved through the scavenging of some deleterious radicals (DPPH, ABTS and OH), the reduction of ferric iron, the total antioxidant capacity determination and the total phenolic, flavonoids, anthocyanins compounds and vitamin C assessment. So the final classification is the following one: *Pentaclethra macrophylla* > *Alchornea cordifolia* > *Panda oleosa* > *Musanga cecropioides* > *Landolphia hispida* > *Ficus benjamina* > *Aframomum melegueta* peelings > *Uapaca staudtii* > *Pseudospondias microcarpa* > *Aframomum melegueta*. All this activities may be relevant for the use of the most actives amongst these fruits as nutraceutical for the management of inflammatory related diseases.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

All procedures in this study followed the Cameroon National Veterinary Laboratory guidelines and the work received the approval of the Animal Ethical Committee of the Laboratory of Animal Physiology of the University of Yaoundé I–Cameroon.

Author Contributions

BEE, SNP, and MLM carried out all experiments reported in the manuscript. BEE, SNP, FNN and PFM designed the study. The final manuscript was read and approved by all authors.

References

- [1] Torres CR, Isla MI, Ríos JL, Giner RM, Alberto MR. Anti-inflammatory properties of hydroalcoholic extracts of Argentine Puna plants. *Food Res Int* 2015; 67: 230–237, <http://dx.doi.org/10.1016/j.foodres.2014.11.012>
- [2] Cholet J, Decombat C, Vareille-Delarbre M, Gaiche M, Berry A, Ogé-ron C, et al. Anti-inflammatory and antioxidant activity of an extract of *Luzula sylvatica*, in a co-culture model of fibroblasts and macrophages. *Curr Res Cmpl Alt Med* 2022; 6(1):152. <https://doi.org/10.29011/2577-2201.100052>
- [3] Turner MD, Nedjai B, Hurst T, Pennington DJ. 2014. Cytokines and chemokines: at the crossroads of cell signaling and inflammatory disease. *Biochim Biophys Acta-Mol Cell Res* 2014; 1843: 2563-2582, doi: 10.1016/j.bbamcr.2014.05.014
- [4] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta-Mol Cell Res* 2011; 1813: 878-888, doi: 10.1016/j.bbamcr.2011.01.034
- [5] Kawara K, Hohjoh H, Inazumi T, Tsuchiya S, Sugimoto Y. Prostaglandin E2-induced inflammation: relevance of prostaglandin E receptors. *Biochim Biophys Acta-Mol Cell Biol Lipids* 2015; 1851: 414-421, doi: 10.1016/j.bbalip.2014.07.008
- [6] Manosroi A, Saraphanchotiwiththaya A, Manosroi J. Immunomodulatory activities of *Clausena excavata* Burm.f. wood extracts. *Journal of Ethnopharmacology* 2003; 89: 155-160, doi: 10.1016/s0378-8741(03)00278-2
- [7] Pei R, Liu J, Martin DA, Valdez JC, Jeffery J, Barrett-Wilt GA, Liu Z, Bolling BW. Aronia Berry Supplementation Mitigates Inflammation in T Cell Transfer-Induced Colitis by Decreasing Oxidative Stress. *Nutrients* 2019; 11: 1316, doi: 10.3390/nu11061316
- [8] Sowjanya KM, Narendra K, Swathi J, Satya K. Phytochemical extraction and antimicrobial efficiency of crude leaf extract of medicinal plant, *Cascabela thevetia*. *International Journal for Research in Pharmacology and Biomedical Science* 2013; 4: 465-470, ISSN: 2229-3701.

- [9] Gothai S, Arulselvan P, Tan WS, Fakurazi S. Wound healing properties of ethylacetate fraction of *Moringa oleifera* in normal human dermal fibroblasts. *Journal of Intercult. Ethnopharmacology* 2016; 5: 1-6, doi: 10.5455/jice.20160201055629
- [10] Matig OE, Ndoye O, Kengue J, Awono A. Edible fruits from cameroon's flora. IPGRI Regional Office for West and Central Africa. 2011. ISBN-13: 978-92-9043-707-9; ISBN-10: 92-9043-707-3.
- [11] Zengin G, Aktumsek A, Guler GO, Cakmak YS, Yildiztugay E. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz. *Records of Natural Products* 2011; 5(2): 123–132.
- [12] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; 26: 1231-1237, DOI: 10.1016/s0891-5849(98)00315-3
- [13] Xiao-Yu S, Zhen-Y UW, Jian-Ren L. In vitro and in vivo antioxidant activity of *Pinus koraiensis* seed extracts containing phenolic compound. *Food Chemistry* 2009; 117: 681-689, <https://doi.org/10.1016/j.foodchem.2009.04.076>
- [14] Narayan S. In-vitro antioxidant activity and free radical scavenging potential of three different extract of leaves of Chinese Ornamental plant. *Asian Journal of Pharmacy and Pharmacology* 2018; 4(2): 159-167; 2(1): 1344–1352, <https://doi.org/10.31024/ajpp.2018.4.2.10>
- [15] Kar B, Kumar RB, Karmakar I, Dolai N, Bala A, Mazumder UK, et al. Antioxidant and in in-vitro anti-inflammatory activities of *Mimusops elengi* leaves. *Asian Pacific Journal of Tropical Biomedicine* 2012; 2(2): 976– 980, doi:10.1016/S2221-1691(12)60346-3
- [16] Lee J, Durst RW, Wrolstad R.E. (2005) Determination of monomeric anthocyanins pigment content of fruits juices, beverages, natural colorants and wines by the pH differential method: collaborative study. *Journal of AOAC International* 2005; 88(5): 1269-1278, PMID: 16385975.
- [17] Devolli A., Stafasani M., Shahinasi E., Dara F., Hamiti H. Determination of vitamin C content in commercial fruit juices by volumetric and spectrophotometric methods. *Journal of Hygienic Engineering and Design* 2021; UDC 664.81:577.164.2: 124-131.
- [18] Dhar P, Tayade AB, Bajpai PK, Sharma VK, Das SK, Chaurasia OP, et al. Antioxidant capacities and total polyphenol contents of hydroethanolic extract of phytococktail from trans-Himalaya. *J Food Sci* 2012; 77(2): C156–C161, doi: 10.1111/j.1750-3841.2011.02523.x
- [19] Titus RG, Gueiros-Filho FJ, de Freitas LA, Beverley SM. Development of a safe live *Leishmania* vaccine line by gene replacement. *Proc Natl Acad Sci U S A* 1995; 92(22): 10267–10271, doi: 10.1073/pnas.92.22.10267
- [20] Bansal SK. Carbohydrate metabolism in the rat peritoneal macrophages. *J Biosci* 1987; 12(1): 415–420, ID: sea-160624.
- [21] Wu J, Zhou J, Chen X, Fortenbery N, Eksioglu EA, Wei S, et al. Attenuation of LPS-induced inflammation by ICT, a derivate of icariin, via inhibition of the CD14/TLR4 signaling pathway in human monocytes. *Int Immunopharmacol* 2012; 12(1): 74–79, doi: 10.1016/j.intimp.2011.10.015
- [22] Sun J, Zhang X, Broderick M, Fein H. Measurement of nitric oxide production in biological systems by using Griess reaction assay. *Sensors* 2003; 3(8): 276–284, <https://doi.org/10.3390/s30800276>
- [23] Suzuki Y, Orellana MA, Schreiber RD, Remington JS. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science* 1988; 240(4851): 516–518, DOI: 10.1126/science.3128869
- [24] Kumari SC, Yasmin N, Hussain RM, Babuselvam M. In vitro anti-inflammatory and anti-arthritic property of *Rhizopora mucronata* leaves. *International Journal of Pharmacological Sciences and Research* 2015; 6(3): 482–485, ID: 73570510
- [25] Zeng Y, Song J, Zhang M, Wang H, Zhang Y, Suo H. Comparison of In Vitro and In Vivo Antioxidant Activities of Six Flavonoids with Similar Structures. *Antioxidants (Basel)* 2020; 9(8): 732, DOI: 10.3390/antiox9080732
- [26] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *Journal of Agricultural Food Chemistry* 2005; 53(6): 1841-1856, DOI: 10.1021/jf030723c
- [27] Foti MC, Daquino C, Geraci C. Electron-transfert reaction of cinnamic acids and their methyl esters with the DPPH radical in alcoholic solutions. *Journal of Organic Chemistry* 2004; 69: 2309-2314, doi: 10.1021/jo035758q

- [28] Pechangou NS, Enang II EB, Ngohoba VS, Njaya EM, Njaya FN, Moundipa FP. Crude extract of *Codiaeum variegatum* stem exhibit potent antioxidant and anti-inflammatory activities in vitro. *Journal of Exploratory Research in Pharmacology* 2022. doi:10.31183/imcp.2023.00070.
- [29] Stagos D. Antioxidant activity of polyphenolic plant extracts. *Antioxidants (Basel)* 2019; 9(1): 19 DOI: 10.3390/antiox9010019
- [30] Antonelli M, Kushner I 2017. It's time to redefine inflammation. *FASEB J.* 31, 1787-1791, doi: 10.1096/fj.201601326R
- [31] Laskin DL. Macrophages and inflammatory mediators in chemical toxicity: a battle of forces. *Chemical Research in Toxicology* 2009; 22: 1376-1385, doi: 10.1021/tx900086v
- [32] Ariel A, Maridonneau-Parini I, Rovere-Querini P, Levine JS, Mühl H. Macrophages in inflammation and its resolution. *Frontier in Immunology* 2012; 3: 324, doi: 10.3389/fimmu.2012.00324
- [33] Huet O, Choukroun G, Mira J-P. Toll like receptor, inflammatory response and sepsis. *Reanimation* 2004; 13: 167–175, doi: 10.1517/14728222.7.5.603
- [34] Ondua M, Njaya EM, Abdalla MA, McGaw LJ. Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. *Journal of Ethnopharmacology* 2019; 234: 27–35, doi: 10.1016/j.jep.2018.12.030
- [35] Bhaskaran N, Shukla S, Srivastava JK, Gupta S. Chamomile: an anti-inflammatory agent inhibits inducible nitric oxide synthase expression by blocking RelA/p65 activity. *International Journal of Molecular Medicine* 2010; 26: 935-940, doi: 10.3892/ijmm.00000545
- [36] Lalles J-P. Intestinal alkaline phosphatase: a very protective enzyme with potent anti-inflammatory properties. *Nutrition and Dietetic Book* 2014; 49: 81-87, doi: 10.1111/nure.12082
- [37] Yougbaré-Ziebroou MN, Ouédraogo N, Lompo M, Bationo H, Yaro B, Gnoula C, et al. Anti-inflammatory, analgesic and antioxidant activities of the aqueous extract of foil rods of *Saba senegalensis* Pichon (Apocynaceae). *Phytotherapies* 2016;14:213–219, doi : 10.9734/ejmp/2019/v27i330116
- [38] Sekhar S, Sampath-Kumara KK, Niranjana SR, Prakash HS. Attenuation of reactive oxygen/nitrogen species with suppression of inducible nitric oxide synthase expression in RAW 264.7 macrophages by bark extract of *Buchanania lanzan*. *Pharmacognosy Magazine* 2015; 11: 283–291, <https://doi.org/10.4103/0973-1296.153080>
- [39] Owona BA, Njaya NF, Laufer S, Moundipa PF, Schluesener HJ. A fraction of stem bark extract of *Entada africana* suppresses lipopolysaccharide-induced inflammation in RAW 264.7 cells. *Journal of Ethnopharmacology* 2013; 149(1): 162–168, doi: 10.1016/j.jep.2013.06.016
- [40] Thomas DC. How the phagocyte NADPH oxidase regulates innate immunity. *Free Radical Biology and Medicine* 2018; 125: 44-52, doi: 10.1016/j.freeradbiomed.2018.06.011
- [41] Radmark O, Werz O, Steinhilber D, Samuelson B. 5-lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease. *Biochimica and Biophysica Acta* 2015; 1851(4): 331-339, doi: 10.1016/j.bbali.2014.08.012
- [42] Valdez J.C., Bolling B.W. (2019). Anthocyanins and intestinal barrier function: a review. *Journal of Food Bioactives*, 5:18-30, <https://doi.org/10.31665/JFB.2019.5175>