Comparative evaluation of the antioxidant and anti-inflammatory properties of *Musa cavendish* and *Musa paradisiaca* pulp and peel extracts from Guinea

Mamady Diawara 1, Imane Boukhers 15, Karine Portet 15, Orianne Duchamp 1, Sylvie Morel 2, Frederic Boudard 1, Lounseny Traore 3, Alain Michel 1, Claudie Dhuique-Mayer 1, Patrick Poucheret 1*

1 Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de La Réunion, Montpellier, France

2 Laboratoire de Botanique, Phytochimie et Mycologie, CEFE, CNRS-Université de Montpellier-Université Paul-Valéry Montpellier-EPHE-IRD, Montpellier, France

3 Laboratoire de Chimie alimentaire, Université Gamal Abdel de Conakry, Guinée,

*Both authors equally contributed

1. INTRODUCTION

Fruits are considered as essential components of a healthy diet because of their content in vitamins, minerals, fibers and bioactive secondary metabolites.

According to literature reports, higher intakes of fruit are associated with lower mortality, and reduced incidence of various chronic diseases including type 2 diabetes and cardiovascular disease 1,2. These results support the current World Health Organization dietary recommendations to increase daily fruits consumption 3.

Banana (*Musa sp.*) is one of the most widely grown tropical fruits in the world. It is both popular and affordable. It is ranked as the fourth most important agricultural product after rice, wheat and corn in terms of world production, with an output of 100 million tons per year. More than 1000 varieties of bananas are produced in the world. The most commercialized variety is *Musa cavendish*, which represents about 45% of the world banana market. The other major group of banana varieties is the *Musa paradisiaca* commonly called plantain, with more than 100 cultivars 4.

Banana fruit is composed of two main parts: the skin and the pulp. Pulp is the edible part. It contains an abundant amount of nutrients: free sugars, total starch but also resistant starch, some essential minerals, such as phosphorus, sodium, potassium, calcium, magnesium, iron, copper, zinc and manganese 5,6. The skin is the first by-product of this fruit. It represents about 40% of the total mass of the fruit and has long been considered as a waste. Until recently, banana peel had no useful application and was landfilled, bringing massive amounts of organic material to be managed. However, since researchers began to focus on studying its composition, several possible applications have emerged, ranging from its use as an ingredient for food fortification in the food industry to the extraction and isolation of functional components. They mainly include: bioactive compounds and secondary metabolites of plants 7-9.
Secondary metabolites are among the most studied phytochemical compounds in fruits and vegetables. They represent a family of organic molecules widely present in the plant kingdom. They are classified into different categories, associated with various properties and effects on human health. This is an area of interest for research on food bioactive compounds, and some research has focused on those present in banana pulp. They indicate the presence of phenolic compounds, such as catechins (epicatechin and gallatechin), ferulic, sinapic, salicylic, gallic acid, carotenoids, flavonoids, biogenic amines including dopamine, phytosterols and a significant amount of ascorbic acid. Bananas have a greater antioxidant potential than various berries, herbs and vegetables, attributed to the prevalence of these components.

As for this fruit peel, it has long been used in traditional medicine for the treatment of anemia, burns or inflammation. Studies report many of its pharmacological properties such as: inhibition of a wide range of bacteria and fungi growth, reduction of blood sugar, inhibition of the development of certain cancer cell types but also antioxidant property which according to some literature reports may be stronger than pulp. Indeed, this by-product contains interesting bioactive compounds such as dopamine, L-dopa, ascorbic acid, rutin, carotenes, tocopherols, catecholamines and phenolic acids.

In addition to the antioxidant effects currently known, the secondary metabolites thanks to their structure diversity and mechanisms of action exert other positive effects on health, including prevention of some cancers, neurodegenerative diseases, cardiovascular diseases, obesity and inflammation.

In Guinea, banana consumption, fresh or cooked, is common to all socio-professional strata and is part of the basic diet. However, the skin is still unexploited and considered a waste product.

In the present study, our approach was to produce dry extracts of pulp and bark of *Musa cavendish* and *Musa paradisiaca* from Guinea, to explore and compare their biological activities *in vitro* by evaluating: their polyphenol content and their antioxidant properties, as well as their anti-inflammatory and immunomodulatory potential.

Our aim was to improve the knowledge of the effects of Guinea banana pulp and peel on health through their ability to influence the levels of oxidative stress and inflammation, and thereby indirectly their nutrition-health potential on chronic diseases.

Such knowledge could open the possibility of using the biological properties of banana agroresource from Guinea for the pharmacological potential of their bioactive content in the management of non-transmissible diseases such as metabolic syndrome.

2. MATERIALS AND METHODS

2.1. Chemicals

Trolox (98%), Na₂HPO₄, 2-amino-ethyl diphenyl borinate and acetic acid were from Fluka Chemicals (Illkirch, France). DPPH and AAPH radicals, chlorogenic acid (95%), cyclohexane (99.8%), acetonitrile, and dimethyl sulfoxide (DMSO; 99.9%) were purchased from Sigma-Aldrich (Illkirch, France). Fluorescein is from Panreac. Ethanol (96%) and methanol (99.9%) were obtained from VWR. Chloroform (99%) and Na₂HPO₄ (99%) are from Honeywell Research Chemicals (Illkirch, France).

RPMI medium 1640 GlutaMAX®, penicillin–streptomycin, murine recombinant interferon γ, Hank’s Balanced Saline Solution (HBSS) and fetal bovine serum were obtained from Gibco. LPS E. coli 055:B5 and sodium nitroprusside were purchased from Sigma Aldrich (Illkirch, France). MTS (3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium) was purchased from Promega (Madison, Wisconsin). PMS (phenazinemethosulfate) was purchased from ICN Biomedical (Aachen, Germany).

2.2. Harvesting bananas and preparing samples

Bananas and plantains were harvested in the field of the Centre de Recherche Agronomique de Kindia (CRAK) in the Republic of Guinea. They were harvested at green stage after 3 months of growth cycle. Upon harvest, they were packed in prepared boxes and shipped to Montpellier within 24 h. In the pharmacology laboratory, bananas were peeled and separated in two batches of respective peel and pulp. Each batch was then freeze-dried, at a temperature of -104°C and under vacuum, to remove the water content of the plant matrix by sublimation without changing its nutrients.

After freeze-drying, the batches of freeze-dried banana peel and pulp are ground, sieved through an 8 mm mesh sieve. The obtained powder was weighed before delipidation. Delipidation was performed in a Soxhlet apparatus as follows. 100 g of banana matrix powder mixed with 2.7 L of hexane in a 5000 mL flask. A cooler was installed above the glass to condensate the hexane vapors. The delipidated product (skins or pulp) was also dried, weighed and submitted to maceration.

The maceration of the delipidated powder allowed to obtain the liquid extract of the Guinea banana matrix. It was carried out as follows. A mixed solution of solvents is prepared and consists of 400 mL of absolute ethanol, 95 mL of distilled water and 5 mL of normal acetic acid. Banana powder (peel or pulp) in the Erlenmeyer flask was suspended in 50 mL of this solvent mixture. The suspension was subjected to a moderate agitation for 24 h for a first maceration. At the end of this time, the maceration process was stopped and the suspension remained at rest for 10 to 15 min. Finally, the supernatants were collected in centrifuge jars. The maceration paste was again suspended in 500 mL in the solvent mixture prepared beforehand for a second maceration of 24 h. The 2 hydroalcoholic phases were then be processed and put back into the same batch.

Following maceration, the supernatant was collected in jars of identical weight to facilitate centrifugation. The supernatants were centrifuged at 3500 rpm during 15 min. The supernatant was then filtered using a vacuum pump. This process allowed to obtain clear hydroalcoholic extracts that were submitted to evaporation to remove ethanol. It was frozen before being lyophilized to produce the dry extract of banana or plantain. The extracts were weighed and stored at minus 80°C until used for analysis.

2.3. TPC (Total Phenolic Content)

The determination of total polyphenols was performed with the Folin-Ciocalteu reagent according to the method adopted in the botany laboratory of the Faculty of Pharmacy, University of Montpellier. The determination of total polyphenols was performed with the Folin-Ciocalteu reagent according to the method adopted in the botany laboratory of the Faculty of Pharmacy of the University of Montpellier. According to this method, the Folin-Ciocalteu reagent (FC) is able to oxidize all phenolic compounds in plant extracts. This Folin-Ciocalteu reagent (FC) is a mixture of phosphotungstic acid and phosphomolybdic acid, which is yellow in color and turns blue when reduced by phenols. Thus, the blue coloration

CODEN (USA): JDDTAO
produced has a maximum absorption around 650 nm and is proportional to the level of phenolic compounds.

The dry extracts of skin and pulp were weighed and diluted in DMSO (dimethylsulfoxide), at 1 mg / mL and passed to ultrasound for 5 min to facilitate its solubilization. From this solution a solution of 0.5 mg / mL was prepared. Distilled water and extracts of pulp and skin without mixing with the 10% Folin-Ciocalteu reagent were considered white during the experiment. Gallic acid was used as a calibration range at dilutions of 1.56; 3.125; 6.25; 12.5; 25; 50 and 75 µg / mL. It was prepared, extemporaneously, 1 mL of a commercial solution of 10% Folin-Ciocalteu in 9 mL of distilled water. Finally, the different solutions were distributed in a 96-well triplicate plate as follows: 50 µL of skin and pulp extracts, 50 µL of distilled water, 50 µL of 10% Folin-Ciocalteu and 50 µL sodium carbonate (Na₂CO₃). The incubation time was 60 min at room temperature, the plate being protected in aluminum foil to avoid the effect of light on the Folin-Ciocalteu. The plate was read by a spectrometer (MDS Inc., Toronto, Canada) and the absorbance was measured at 650 nm. Results are expressed in microgram gallic acid equivalent per gram of fresh banana or plantain in (mg GAE / g fw).

2.4. Characterization of the antioxidant power of Musa cavendish and plantain extracts in vitro

2.4.1. DPPH method

DPPH is an anti-free radical test method used by several researchers, used here according to the method described by Morel et al. 27. The dry extracts of skin and pulp were weighed and dissolved in DMSO, at 1 mg / mL. This solution was sonicated for 5 min to facilitate its solubilization, then diluted 1/2 in absolute ethanol. Ethanol and extracts (pulp and skin) without DPPH were used as blank. The trolox was used as a calibration range at concentrations of 12.5, 25, 50 and 75 µM. Ethanolic rosemary extract (i.e. 0.2 mg of ethanolic extract of rosemary diluted in 1 mL of ethanol) and chlorogenic acid at 0.01 mg / mL of ethanol were used as positive controls. In a 96-well plate, 100 µL of the range, chlorogenic acid, rosemary, and skin and pulp extracts were dispensed for the different concentrations to be tested. Finally, 75 µL of absolute ethanol and 25 µL of the DPPH solution (ie 2 mg in 5 mL of ethanol) prepared immediately were also added in order to start the reaction and bring the final volume to 200 µL. It should be noted that the DPPH had been stirred for 1 h in the dark and filtered using a 0.45 µm nylon filter, hydrophobic, 25 mm in diameter. Each solution was distributed 3 times in the wells according to the plate plan. The plate was protected from light with aluminum foil and incubated for 30 min at room temperature. Absorbance was measured at 550 nm using a microplate reader. Results are expressed in micromoles of Trolox equivalent per µgram of dry extract (µmol TE / µg extract).

2.4.2. ORAC method

The ORAC assay was performed according to the method described by Morel et al. 27. The extracts were dissolved in DMSO (dimethylsulfoxide) at 1 mg / mL. From this stock solution, a 50 µg / mL solution was prepared in a 75 mM phosphate buffer solution with a pH = 7.4. The trolox used for the calibration range was diluted to 6.25; 12.5; 25; 50 and 75 µM. Chlorogenic acid (8.8 µM) and ethanolic rosemary extract (12.5 µg / mL) served as a positive control. In an opaque 96-well plate, 20 µL of the trolox range at the various concentrations mentioned above were distributed, along with the chlorogenic acid (8.8 µM), the ethanolic extract of rosemary (12.5 µg / mL) and extracts of the skin and pulp at 50 µg / mL. Also, 100 µL of phosphate buffer and 100 µL of freshly prepared 0.1 µM fluorescein solution were added in the phosphate buffer prepared for this purpose. All these solutions were deposited in the opaque triplicate plate. After this phase, the plate was incubated at 37 ° C for 10 min with shaking. The reaction was initiated by the addition of 50 µL of the AAPH (2,2'-Azobis (2-amidinopropane) dihydrochloride) prepared at the time. Fluorescence was then measured and recorded at the excitation wavelength of 485 nm and an emission wavelength of 535 nm, every 5 min for 70 min using a TriStar LB 941 multimode microplate reader. (Berthold Technologies, Bad Wildbad, Germany). Final ORAC values were calculated using a regression equation between Trolox concentration and area under the fluorescein decay curve. Results are expressed in micromoles of Trolox equivalent per µgram of dry extract (µmol TE / µg extract).

2.4.3. Mitotracker method

The use of Mitosox red to detect reactive oxygen species (ROS) is widespread and gives well interpretable results. It is a fluorescent probe that allows the detection of reactive oxygen species by live cell imaging.

Once the probe is introduced into living mitochondrial cells, the positively charged lipophilic triphenylphosphonium portion of the probe oxidizes to dihydroethidium or hydroethidine which has an affinity for superoxide anion. This oxidation gives a red color to the intracellular space in function. This is an indicator of the presence of ROS and depends on the concentration present in the medium.

Our experimentation was carried out following the method developed in the laboratory of Pharmacology of the University of Montpellier. 28. Mitosox was stored at -18°C protected from light to avoid any oxidation process before use. It was tested at 5 mM and the incubation with cells lasted 10 min at 37°C (protected from light); whereas mitotracker was tested at 150 nM and the incubation was performed in 30 min at 37°C. Prior to pretreatment with cavendish and plantain pulp extracts (1mg/mL), cells were placed in transparent 24-well plates and stimulated with LPS/IFN. Stimulated control cells were treated only with LPS/IFN, control cells were not treated with either LPS/IFN or pre-treatment but were adhered to the plates under the same conditions as the experimental cells. Note that this experiment was performed 3 times to confirm the antioxidant effect by labeling the mitochondria. Observation and imaging is performed with a fluorescence microscope and the fluorescence intensity is quantified with the image].

2.5. Anti-inflammatory Activity

2.5.1. Culture cellulaire

The macrophage cell line J774.A1 (ATCC, TIB67) was purchased from LGC Standards (Manchester, NH, USA). Cell culture was performed in RPMI 1640 GlutaMAX® medium supplemented with streptomycin (100 µg/mL) and penicillin (100 units/mL), 10% inactivated fetal calf serum (complete RPMI medium). Cells were incubated at 37°C, 5% CO₂ and 95% humidity.

2.5.2. Dosage of NO (Nitric Oxide), NO scavenging, TNF-α (Tumor Necrosis Factor Alpha) and IL-6 (Interleukin-6)

Macrophage J774.A1 cells were seeded in a 24-well culture plate with complete RPMI medium. The cells were pretreated with different concentrations of Guinea banana extracts with the respective doses of 25, 50, and 100 µg/mL for 4 h and stimulated with LPS (100 ng/mL) and interferon γ (10 ng/mL), and then incubated for 16-18 h at 37°C. Supernatants were collected for nitrite determination or stored at 80 °C until used for NO, TNF-α, and IL-6 assays.

2.6. Statistical analyses
Values are presented as mean ± standard error of the mean (SEM). Statistical analysis of the data was carried out using Prism® software by two-way ANOVA followed by Bonferroni post-test. P values < 0.05 were considered to be significant.

3. RESULTS

3.1. Extraction and extraction performance of samples

The results of extraction yields are shown in Table 1. They indicate that for fresh weights varying from 557.4 to 949.1 g, the quantity of extract obtained was from 6.1 to 9.2 g with extraction yields varying around 1%. It can be observed that the weights of the dry extracts are low compared to the fresh weights. These results are related to the low proportions of dry matter in sweet bananas (7.7% to 12.9%) and in plantains (12.6% to 18.7%) 30. In addition, the peel, which represents only 40% of the dry matter, is reported to contain 89.45% water 30.

Table 1. Results of the extraction yields of dry samples of pulp and skin of Cavendish banana and Guinea plantain. Extraction yields are obtained by taking the ratio of the weight of the dry extract obtained by freeze-drying, delipidation and maceration of the fresh plant matrix batch and the weight of the fresh skin or pulp material, all expressed in grams. The results of these yields are reported as a percentage (%). MC= Musa cavendish ; MP= Musa paradisiaca (plantain).

<table>
<thead>
<tr>
<th>Nº</th>
<th>Cultivars</th>
<th>Extracts</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Extract yield (R %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Musa cavendish</td>
<td>Skin MC</td>
<td>949,1</td>
<td>9,2</td>
<td>0,96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MC</td>
<td>583,6</td>
<td>6,4</td>
<td>1,09</td>
</tr>
<tr>
<td>2</td>
<td>Musa paradisiaca</td>
<td>Skin MP</td>
<td>574,6</td>
<td>7,01</td>
<td>1,21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MP</td>
<td>557,4</td>
<td>6,1</td>
<td>1,09</td>
</tr>
</tbody>
</table>

3.2. Determination of total polyphenols (TPC)

The results are expressed as gallic acid equivalent and represented in Table 2. The values noted show that at 1 mg/mL, all extracts have a lower content than the control (rosemary ethanolic extract).

Plantain peel extract showed the highest polyphenol content (0.42 mg GAE/g), followed by cavendish banana peel extract (0.29 mg GAE/g) which did not show significant difference with cavendish banana pulp extract (0.25 mg GAE/g). Plantain pulp gave the lowest polyphenol content (0.17 mg GAE/g) but statistically, it does not show significant difference with the polyphenol content of cavendish banana peel (0.29 mg GAE/g) and pulp of the same cultivar (0.25 mg GAE/g).

Table 2. Results of the total polyphenols determination. These values represent the averages of 3 replicates of total polyphenol contents in pulp and skin. The total polyphenol contents obtained are expressed in mg GAE/g fw. TPC= Total polyphenol Content, GAE= Gallic acid equivalent, fw= fresh weight. Bars with different letters indicate significantly different levels (P<0.05). Each value represents the mean ± SEM of three trials.

<table>
<thead>
<tr>
<th>Nº</th>
<th>Cultivars</th>
<th>Extrait</th>
<th>TPC (mg GAE/g fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Musa cavendish</td>
<td>Skin MC</td>
<td>0,29 ± 0,03 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MC</td>
<td>0,25 ± 0,02 bc</td>
</tr>
<tr>
<td>2</td>
<td>Musa paradisiaca</td>
<td>Skin MP</td>
<td>0,42 ± 0,05 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MP</td>
<td>0,17 ± 0,02 c</td>
</tr>
<tr>
<td></td>
<td>Ethanol extract of rosemary</td>
<td></td>
<td>56,65 ± 3,68 a</td>
</tr>
</tbody>
</table>

3.3. Antioxidant activity

As previously explained, the evaluation of antioxidant activities of agroresource extracts and food extracts is confirmed by combining the responses of different and complementary tests to get a more accurate idea of the antioxidant capacity of the test sample 31. In this case, DPPH, ORAC and mitotracker tests were combined.

3.3.1. DPPH method

Results of the DPPH assay, presented in table 3, are expressed as µmol TE/g dw. They showed that at 1 mg/mL, all Guinea banana peel extracts showed slightly better antioxidant values than pulp extracts for both varieties. However, in comparison with rosemary extract (internal assay reference) and according to the DPPH method, all extracts gave relatively low antioxidant values. The DPPH results show that plantain peel has the highest free radical activity (40.39 µmol TE/g), but with no significant difference from cavendish banana peel (33.93 µmol TE/g). Also the cavendish banana peel would have statistically similar antioxidant activities (33.93 µmol TE/g) to the pulp of the same cultivar (27.37 µmol TE/g). On the other hand, the antioxidant activities of cavendish banana peel would be better (33.93 µmol TE/g) than those of plantain pulp, which gave the lowest DPPH value (18.96 µmol TE/g). Statistical analyses also show that the pulps of the two cultivars, cavendish banana and plantain, have statistically the same antioxidant activity (18.96 µmol TE/g and 27.37 µmol TE/g) and that the plantain peel would have highly significant antioxidant effects to those of the pulp of the same cultivar (respectively 40.39 µmol TE/g and 18.96 µmol TE/g).
Table 3. Results of the DPPH method. These DPPH values represent the averages of 3 evaluations of the antioxidant activity of Guinea banana pulp and peel. The total polyphenol contents obtained are expressed in µmol TE/g dw. MC = Musa cavendish; MP = Musa paradisiaca (plantain), dw = dry weight, TE = Trolox equivalent, Bars with different letters indicate significantly different levels (P<0.05). Each value represents the mean ± SEM of three trials.

<table>
<thead>
<tr>
<th>№</th>
<th>Cultivars</th>
<th>Extracts</th>
<th>DPPH (µmol TE/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Musa cavendish</td>
<td>Skin MC</td>
<td>33.93 ± 4.93 bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MC</td>
<td>27.37 ± 2.47 cd</td>
</tr>
<tr>
<td>2</td>
<td>Musa paradisiaca</td>
<td>Skin MP</td>
<td>40.39 ± 1.88 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MP</td>
<td>18.96 ± 2.09 d</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract of rosemary</td>
<td></td>
<td>73.81 ± 4.39 a</td>
</tr>
</tbody>
</table>

3.3.2. ORAC method

The results expressed as µmol TE/g dw shown in Table 4, noted that at 1 mg/mL all Guinea banana peel extracts showed slightly better antioxidant values than the pulp extracts for both varieties. However, when compared to the rosemary extract and using the ORAC method, all extracts gave relatively low antioxidant values.

Considering the ORAC results, presented in table 4, they also indicate that plantain peel would have the highest antioxidant capacity (1.36 µmol TE/g). Cavendish banana peel has the same antiradical activity as the pulp of the same cultivar (0.55 µmol TE/g for each type of extract). Cavendish banana peel and pulp would have statistically similar free radical scavenging activities to plantain pulp (0.55 µmol TE/g for cavendish banana peel and pulp and 0.34 µmol TE/g). The ORAC results corroborate those of the DPPH method. Overall, the results are in agreement with previous information related to some limiting factors of the two methods such as: the sensitivity to light, the selectivity of bioactive molecules and the process of interference which is not to be neglected within the extracts. Finally, these results are consistent with the results of the determination of polyphenols by the Folin-Ciocalteu (FC) method.

Table 4. Results of the ORAC method. These ORAC values represent the averages of three antioxidant activity assessments of Guinea banana pulp and peel. The total polyphenol contents obtained are expressed in µmol TE/g dw. MC = Musa cavendish; MP = Musa paradisiaca (plantain), dw = dry weight, TE = Trolox equivalent, Bars with different letters indicate significantly different levels (P<0.05) Rosemary (R) is used as a reference control in all tests (a, b, c; p < 0.05). Each value represents the mean ± SEM of three trials.

<table>
<thead>
<tr>
<th>№</th>
<th>Cultivars</th>
<th>Extracts</th>
<th>ORAC (µmol TE/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Musa cavendish</td>
<td>Skin MC</td>
<td>0.55 ± 0.08 c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MC</td>
<td>0.55 ± 0.05 c</td>
</tr>
<tr>
<td>2</td>
<td>Musa paradisiaca</td>
<td>Skin MP</td>
<td>1.36 ± 0.07 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp MP</td>
<td>0.34 ± 0.005 c</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract of rosemary</td>
<td></td>
<td>2.44 ± 0.14 a</td>
</tr>
</tbody>
</table>

3.3.3. Mitotracker method

The results obtained with mitochondrial marking are presented in Figure 1. The presented test was performed on Musa cavendish and Musa paradisiaca pulp extracts.

Figure 1. Effect of pulp banana at different concentration 75 µg/mL on J-774 macrophages cells mitochondria versus control cells (C) and stimulated cells (LPS/IFN). Each value represents the mean ± SEM of three trials. Pulp MC = Pulp of the Musa cavendish; Pulp MP = Pulp of the Musa paradisiaca
The graph represents the level of superoxide anion (SOA) production per mass of mitochondria in stimulated macrophages pretreated with the extracts at a concentration of 75 µg/mL.

At the concentration tested, pulp samples of both banana varieties significantly inhibited superoxide anion production compared to untreated stimulated macrophages. The results also indicate that the MP pulp extract is slightly more active than the MC pulp extract and the ratio representing superoxide anion production after pretreatment with this extract is similar to the amount produced in control cells.

3.4. Anti-inflammatory activity

3.4.1. Nitric Oxide (NO) production

The production of nitric oxide (NO) by macrophage cells stimulated and treated with banana extracts is presented in Figure 2. Pulp extracts of the 2 varieties induced a similar dose-dependent (not statistically different) inhibition of NO production.

![Figure 2](image1.png)

**Figure 2.** Nitric oxide effect of banana from Guinea. Each value represents the mean ± SEM of three trials. Pulp MC= Pulp of the *Musa cavendish*; Pulp MP= Pulp of *Musa paradisiaca*.

It appears from this histogram that banana and plantain pulp extracts at 100, 50 and 25 µg/mL showed an anti-inflammatory effect when the cells were pretreated for 4h with a 24h stimulation time. This anti-inflammatory effect is clearly visible according to the doses of banana and plantain extract tested.

3.4.2. Nitric Oxide (NO) scavenging

To further explore the potential bioactivity of Guinea banana pulp extracts (*Musa cavendish* and plantain) against the free radical NO, the capacity of Guinea banana extracts to scavenge NO was evaluated. Results are presented in Figure 3

![Figure 3](image2.png)

**Figure 3.** Nitric oxide scavenging effect of banana from Guinea. Each value represents the mean ± SEM of three trials. Bars with different letters indicate significantly different contents (P<0.05); Pulp MC= Pulp of the *Musa cavendish*; Pulp MP= Pulp of *Musa paradisiaca*.

This test indicates that banana pulp extracts have a dose-dependent NO scavenging capacity (statistically different according to the doses and the nature of the plant matrix). However, there is no significant difference between the different types of extract except at 25 µg/mL.

3.4.3. Cytokines: Tumor necrosis factor alpha (TNF-α) et Interleukin-6 (IL-6)

Regarding the cytokines of inflammation, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), their production by stimulated macrophage cells and treated with the pulp and peel extracts of both varieties of Guinea banana was not inhibited (results not shown). Furthermore, none of the extracts, even at 100 µg/mL, showed immunomodulatory or immunostimulatory effects. This result indicates the absence of effect of these extracts on our stimulated inflammatory macrophage cells.

4. DISCUSSION

In experimental work, the extraction phase of biomolecules requires the use of solvents that differ due to their polarity. An ideal solvent should have the following desirable characteristics: have a high solute separation capacity, be selective, dissolve the specific component to a large extent while having a minimum capacity for other components and it must be chemically stable. Then it must be regenerable and it must have a low viscosity to facilitate pumping and transport. Its polarity is manifested by a permanent electric dipole in their molecules, because its atoms have different electronegativities. Finally, the extraction performance of
compounds with antioxidant activity such as polyphenols is generally influenced by the conditions under which the liquid-solid extraction process takes place. The present work in its extraction phase has combined polar and nonpolar solvents at different levels. Our extraction results give quantities of extract which vary from 6 to 9 g for weights of fresh material varying from 557.4 g to 949.1 g, or 1% to 2% of average extraction yield. The relatively low amounts of extract compared to fresh weight would be linked to the very low proportion of dry matter in fresh bananas and plantains. Skin represents 40% of the total fresh material. Our results are similar to some previously published results that found 3.07 g with 300 g of fresh skin, a yield of 1%. Note that this team boiled the banana peels in 900 mL of distilled water for 5 min and then the peel was homogenized and the extraction was carried out with water at 90°C for 2 h. This temperature in this work could have impacts on phenolic compounds such as the hydroxycinnamic acid and flavonol in the dry extract because their storage temperature would be 80°C for 3 years. The solvents used by Someya et al. were chloroform, ethyl acetate and distilled water. We dried our hydroalcoholic liquid extract to obtain the dry extract in the same way as the ethyl acetate phase of Someya et al. by freeze drying. The use of ethanol in our study has advantages for the extraction of antioxidant compounds, as it is inexpensive, reusable, non-toxic, and the extracts can be used in the food industry. This solvent has “GRAS” status (generally recognized as safe according to the American Food and Drug Administration). The acetic acid used has some action a breaking up the polyphenols and releasing biomolecules as demonstrated in several studies. It could also protect the material against oxidation. It is therefore important to acidify solvents with organic acids (formic or acetic acid) rather than with mineral acids such as 0.1% HCl. Water has been combined with acetic acid to aid in the extraction of chemicals soluble in water and/or organic solvents. Finally, it should be noted that there is no universal extraction method applicable to the extraction phenolic compounds from plant materials. This is due to the complexity of phenolic compounds and their interaction with other known bioactive molecules in plant matrices.

Bananas contain bioactive compounds with antioxidant potential that contribute to physiological defense against oxidative reactions and mediation of free radicals in biological systems. Among the bioactive phytochemicals, polyphenols have been widely cited and studied as the most abundant antioxidants provided by the human diet. They may exert a wide range of biological activities, including antioxidant and anti-inflammatory properties, associated with obesity and chronic inflammation. It should be noted that the Folin reagent used for the determination of polyphenols, can react with other molecules such as nitrogen compounds, thiols, vitamins, nucleotide bases, and carbohydrate bases. In addition to this, the extraction time or the ambient temperature used during the extraction may also influence the reaction. Knowing that delipidation was intended to avoid this phenomenon of reaction or interference with lipids, this finding could be attributed to the existence of different molecules such as proteins, vitamins, starch and lipids that can react with the Folin-Giochaltu (FC) reagent.

Tropical fruits such as citrus, pineapple, papaya, banana, etc. are recognized as potential sources of polyphenols, but due to various resource limitations, their biological capacity is still poorly studied. The total polyphenol content of agroecological zones, assay methods using several polar and nonpolar solvents in various laboratories. Some authors argue that polyphenol contents may also depend on the ripening level. According to them, ripe fruits are richer in phenolic than the pulp. They obtained a TPC content of Nipah banana pulp from Malaysia (0.36 mg GAE / mg fw) higher than the pulp of cultivar Mas (0.32 mg GAE / mg fw), as well the banana peel of Awak (0.48 mg GAE / mg fw) would have the same polyphenol content as the skin of the Guinean plantain (0.42 mg GAE / mg fw). All of these bananas are cultivars from Malaysia. Guinea cavendish banana peel has the same mean TPC value as Brazilian cavendish banana flour (0.29 mg GAE / mg fw) tested in vitro. In accordance with several studies on different banana cultivars indicating that the peels are richer in polyphenol than the pulp, the peels of Guinean plantain demonstrated higher total polyphenol content (0.42 mg GAE / mg fw) compared to other extracts. However, the difference remains negligible between the skin and the pulp of cavendish bananas (0.29 mg GAE / mg fw and 0.25 mg GAE / mg fw for the pulp). This approximation of TPC contents was also observed in another study. In this study carried out on the cultivar Kapas, they found 0.06 mg GAE / mg fw for the skin and 0.05 mg GAE / mg fw for the pulp. These contents are also very low compared to our results. Furthermore, the pulp of Guinean cavendish banana has lower polyphenol content (0.25 mg GAE/g) when compared to Musa cavendish from Nigeria (0.94 mg GAE/g). On the other hand, they obtained a TPC content of Nipah banana pulp from Malaysia (0.36 mg GAE / mg fw) higher than the TPC value of its skin (0.29 mg GAE / mg fw). Finally, their lyophilized extract of Raja purple gave the highest content of multiple peel extracts (76.37 mg GAE / mg fw). This strongly suggest that the pulp could have a polyphenol content similar to the skin or higher, although the latter was not observed in our experiments. These results can be linked to several factors, the main ones being the variety or cultivar, agroecological zones, assay methods using several polar and non-polar solvents in various laboratories. Some authors argue that polyphenol contents may also depend on the ripening level. According to them, ripe fruits are richer in phenols and stage 5 of ripening with the yellow-green color would be very rich in phenolic compounds. The same source reports that the peel would often have a higher polyphenol content than the pulp. It should also be noted that certain compounds arise during the ripening process, coming from plant by-products such as banana peels due to their high enzymatic activity. On the other hand, Folin would have reacted with other non-polyphenolic biomolecules, thus modifying the TPC contents either in the skin or in the pulp. Finally, previous studies have shown that the polyphenol content of the pulp meal stripped of its skin could be between 11.8 to 90.4 mg of GAE / 100g fw. This interval does contain
the range of mean TPC values of banana and guinea plantain (17 mg GAE / 100g to 25 mg GAE / 100g fw).

The DPPH values indicating the antioxidant activities of our extracts range from 18.96 µmol TE / g dw to 40.39 µmol TE / g dw. These values are much higher than those of Costa Rican green banana flour (2.48 µmol TE / 100g dw, or 0.024 µmolTE / g dw) reported by other scientific research 57. This publication indicates that the free radical scavenging activity of this banana is related to the total bound and free polyphenols in its native flour. In comparison to our results, much higher antioxidant activities of *Musa paradisiaca* (plantain) have been obtained by other researchers, i.e. 33.625 µmol TE / g dw 58. It should be noted that their samples were collected from a village in the region of India and the samples were extracted with 70% acetone. In the same study, it was noted that wild banana species have higher free radical scavenging activity than commercial species (420194.7 µmol TE / g extract for wild species) identified in the same agroecological zone. On the other hand, green banana peels (*Musa* sp., Group AAB, Subgroup Prata), treated with ethylenediamine gave on average a DPPH value equal to 380.37 µmol TE/g dw 7, significantly higher than Guinea bananas. Following the study conducted by a research team on banana and plantain genotypes for biofortification purposes, mainly due to the content of resistant starch (RS) and polyphenols, the DPPH value of the Tiparot genotype after cooking for 10 min in 300 mL of water, was 999.9 mg TE / g dw, or 39.94 µmol TE / g dw 59. This result obtained from the pulp of this genotype is similar to the DPPH value obtained from the skin of the guinea plantain (40.39 µmol TE / g dw) statistically equal to the DPPH value from the cavendish banana peel obtained from Guinea (33.93 µmol TE / g dw), which means that they have similar antioxidant activities. Just as the pulp of cavendish bananas from Guinea would have the same anti-free radical activity as the pulp of fresh pulp of the Ney Poovan genotype, ie 27.37 µmol TE / g and 28.51 µmol TE / g respectively. The skin of the cavendish banana and the pulp of the Guinea plantain exhibit free radical scavenging capacities (DPPH = 33.93 µmol TE / g and 18.96 µmol TE / g, respectively) similar to those of the pulp of the genotype Simili Rajash (84.48 µmol TE / 100g, or 33.74 µmol TE / g) and of the Pepita genotype (448 mg TE / 100g, or 17.89 µmol TE / g). Our extracts exhibited antioxidant activities that were all greater than that of the pulps of Angolan plantains after cooking in 300 mL of water for 10 min (295.8 mg TE / 100g or 11.81 µmol TE / g).

On the other hand, the ORAC method was combined with the DPPH method to assess the antioxidant activities of our various extracts. The ORAC method confirmed that plantain peel has better antioxidant activity compared to other extracts and its pulp has less free radical scavenging power. However, it should be noted that our results are still expressed in µmol TE / g of extract, the ORAC values (from 0.34 to 1.36 µmol TE / g of dry extract) are very small compared to the values of DPPH which vary between 18.96 to 40.39 µmol TE / g of extract. This may be linked to a phenomenon of interference from macromolecules such as proteins in ORAC test 59.

Our ORAC results are lower than the value reported by Floegel et al. 59, of 879 µmol TE / 100 g or 8.79 µmol TE / g for bananas consumed in America. This research aimed to know the antioxidant capacities of 50 fruits including bananas, vegetables and drink in the American diet. In their study, the banana samples were washed, dried, chopped and extraction was done with 80% absolute methanol before free radical testing. In our research, the guinea plantain peels often intended for animal feed and the American watermelon tested by this team, have similar antioxidant capacities (i.e. the respective ORAC values of 1.36 µmol TE / g and 1.42 µmol TE / g dw). When we compare our ORAC values to those of unripe banana peel flour of the Nanicão variety 60 and the test of the supernatant derived from the Nan-wa banana 61, we first find that these 2 agroresources have the same anti-free radical activity (ORAC = 2.61 µmol TE / g of dry extract for each variety), which is higher than our results. Our results are even much lower than the average value found for unripe cavendish banana peel from Brazil, tested by Rebello and colleagues, i.e. 43.55 µmol TE / g dw 62. Authors worked on the anti-free radical potential of 100 foods consisting of fresh fruits and vegetables, nuts, cereals, drink etc 62. The team used the ORAC method by separately determining the antioxidant capacity of lipophilic and hydrophilic phenolic compounds and then calculating the total ORAC value for each food. They revealed that the antioxidant activity of the lipophilic compounds contained in the banana was lower (0.66 µmol TE / g dw) than that of the hydrophilic compounds (8.13 µmol TE / g dw), and the cumulative of the 2 fractions of lipophilic and hydrophilic compounds gave 8.79 µmol TE / g dw. The skin and pulp of cavendish bananas from Guinea have free radical scavenging capacities similar to that of lipophilic compounds from bananas sampled by Wu et al. 62.

Overall, the results of the antioxidant activity test by the DPPH and ORAC methods indicate that the cavendish banana and plantain from Guinea have antioxidant activities proportional to their polyphenol content as confirmed by several authors 63,5. The higher the content of the plant and food matrices are in polyphenols, the greater their antioxidant activity. The DPPH test indicated that plantain peel statistically has the same antioxidant capacity (40.39 µmol TE / g dry extract) as cavendish banana peel (33.93 µmol TE / g dry extract) and they are statistically superior to the other extracts for the 2 cultivars.

Using the method of detecting reactive oxygen species in cell membranes by imaging, cavendish and Guinea plantain pulps were modestly effective in protecting cells from free radicals. This was objectivized by the slight change in cell structure and the presence of red spectra when MitoSox red was used at 5 µM 63. These authors recommend the use of MitoSox red at 60 µM to detect ROS, following the oxidation of MitoSox giving red fluorescence spectra are formed 64,65. It must be said that we did not find any papers better detailing the specific case of banana, let alone studies on Guinea banana.

Based on the results of polyphenols dosage and antioxidant tests, the anti-inflammatory activity started with the hydroalcoholic extracts of banana peel. According to several studies in phase with our results above, the banana peel would be richer in phytoconstituents than the pulp. By different phytochemical testing methods, it was proven that banana peel would have higher antioxidant, anti-inflammatory, analgesic, etc. activities than the pulp. These biological properties would be related to the nature of the bioactives in the plant matrix, the level of fruit maturity, the extraction techniques, etc 64,65. Guinea banana peel extracts for both varieties did not show any anti-inflammatory effect despite the different evaluation methods used. These extracts do not stop the production of NO (nitric oxide). However, ethanolic extracts administered orally to rats at doses of 200 mg/Kg and 400 mg/Kg of banana peel extracts showed an anti-inflammatory effect and the 400 mg/Kg dose gave the best result (63% inhibition in 6h). In this experiment, water was used as negative control (10 mL/Kg) and diclofenac sodium (10 mg/Kg) as positive control 67. The inactivity of banana peel extracts could be related to the extraction technique, extract doses, banana varieties and growing conditions, etc [68]. In banana, polysaccharides are the predominant bioactive components 69,70. Starch, one of the most important polysaccharides in banana pulp, is a polymeric mixture of...
glucose, amyllose and amylopectin polymers. Its proportion in the banana peel would be very low compared to the pulp. Other polysaccharides such as homologalacturonans, arabinogalactan, rhamnogalacturonan and mannan, have already been identified in banana pulp with several health effects. The α-D-[1→6]-glucan could significantly promote regulatory activities on the production of nitric oxide (NO), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Pulp extracts inhibited NO production in a dose-dependent manner. This dose-dependent inhibition effect for the 2 varieties remained low (not reaching 50%). This dose-dependent inhibition of NO production is consistent with multiple studies of agricultural products of tropical origin with proven anti-inflammatory activities. The ability of Guinea banana pulp extracts to scavenge free radicals could be related to macromolecules such as polysaccharides. These biomolecules have the ability to inhibit NO production with a dose-dependent effect. In green bananas, there are important polysaccharides such as arabinogalactan. The production of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) by macrophage cells stimulated and treated with guinea banana pulp and peel extracts was not inhibited. On the other hand, during the TNF-α assay, a slight stimulation of macrophage cells was observed. None of the extracts even at 100 μg/mL, showed immunomodulatory or immunostimulatory effects with respect to the production of the cytokine interleukin-6 (IL-6). This indicates the complete inactivation of bioactive in these extracts. We believe that these results are related to the hydroalcoholic nature of our extracts. Banana is a source of nutrients and bioactive molecules including carotenoids, phenolic compounds, vitamins and minerals, vitamins and minerals. These compounds are the source of several beneficial effects for the health of consumers. The above in vitro techniques can identify its nutritional and health profile. The results obtained from the effect of the extracts on the production of pro-inflammatory cytokines are not in accordance with the research work indicating that a dietary intervention with green dwarf banana flour (5% or 10%) would increase the concentration of acetate, propionate and butyrate. This was able to prevent relapse of inflammatory bowel disease symptoms. It also demonstrates that the route of administration would affect the efficacy of bioactive in an agroresource. In another study, banana pulp extracts led to a marked spreading of macrophages depending on the cell strains. It was found in the same study the induction of TNF-α depending on the maturity and variety of bananas. On the other hand in another scientific research, it is mentioned that no investigation on the immunomodulatory activity of banana polysaccharides was reported.

5 CONCLUSIONS
Overall, our results indicate that the peels of both banana varieties were found to be more free radical scavenging than the pulps. Plantain peel performed better with both DPPH and ORAC methods. The Mitotrackr method demonstrated that pulps of Guinea bananas decreased superoxide anion levels in inflammatory cells. A dose-dependent inhibition of NO production and NO scavenging was observed with Guinea banana pulp extracts although it remains moderate. These results indicate an anti-inflammatory property of the extracts. In contrast, proinflammatory cytokines (TNF-α and IL-6) were not inhibited by Guinea banana extracts. Therefore, we suggest to perform further studies focusing on other nature of banana extract to understand the difference of bioactivity potential between cavendish banana and plantain on the one hand, and between peel and pulp on the other. Therefore further studies could help understand Guinea banana extracts absence of activity on specific inflammation mediators and to know the biomolecules responsible for the antioxidant activity and the inhibition of nitric oxide (NO) inflammation marker.

Funding: This research was funded by the French Embassy in Guinea through its Service de coopération et d’action culturelle (SCAC).

Acknowledgments: We thank the French Embassy in Guinea, Mabity Mara, Sylvie Munier, Manon Vitou, Bouri Samba, Alpha Kabinet Keita and Lanceli Kaba for their administrative and scientific support.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References


36. Ngo, T. V.; Scarlett, C. J.; Bowyer, M. C.; Ngo, P. D.; Vuong, Q. V. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis J Food Qual 2017; 9305047. https://doi.org/10.1115/2017/9305047

103(3):1003-1008. https://doi.org/10.1016/j.foodchem.2006.08.038

47. Chen, G. L.; Chen, S. G.; Zhao, Y. Y.; Luo, C. X.; Li, J.; Gao, Y. Q. Total phenolic contents of 33 fruits and their antioxidant capacities before and after in vitro digestion. Ind Crop Product 2014; 57:150-157. http://dx.doi.org/10.1016/j.indcrop.2014.03.018

48. Méndez, C.; Forster, M. P.; Rodríguez-Delgado, M. A.; Rodríguez-Rodríguez, E. M.; Romero, C. D. Content of free phenolic compounds in bananas from Tenerife (Canary Islands) and Ecuador. Europ Food Res Technol 2003; 217:287-290. https://doi.org/10.1007/s00217-003-0762-8


