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Microscopic Characteristics, Chromatographic Profiles and Inhibition of Peroxidase Activity of the Leaves of *Manihot esculenta* and *Manihot glaziovii*, Consumed as Traditional Vegetables

Paulin Mutwale Kapepula[1*], Patricia Mbombo Mungitshi[1], Dieudonné Tshitenge Tshitenge[1], Thierry Franck[2], Dieudonné Mumba Ngoyi[3], Pascal Dibungi T. Kalenda[1], Monique Tits[4], Michel Frédérich[4], Nadege Kabamba Ngombe[1], Ange Mouithys-Mickalad[2]

[1]Centre d'Etudes des Substances Naturelles d'Origine Végétale (CESNOV), Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
[2]Centre for Oxygen Research and Development (CORD), University of Liège, Liège, Belgium
[3]Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
[4]Laboratory of Pharmacognosy, Center for Interdisciplinary Research on Medicines (CIRM), University of Liège, Liège, Belgium
Email: *Garaphmutwal@yahoo.fr

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Abstract

Methanolic extracts from the leaves of *Manihot esculenta* (Two cultivars) and *Manihot glaziovii*, consumed as traditional vegetables in DR. Congo was chemically characterized by Thin layer Chromatography and High Performance Liquid Chromatography. *In vitro* biochemical activities of extracts against Radical Oxidative Species (ROS) production were assessed in cellular models, on enzymes, Myeloperoxidase (MPO) and Horseradish Peroxidase (HRP) involved in inflammation. The microscopic analysis of the powder of leaves showed that each species displays specific and discriminating botanical microscopic features. Varieties of *M. esculenta* had a chemical fingerprint different from *M. glaziovii*. The majority of compounds were polyphenols, represented mainly by rutin, kaempferol-3-O-rutinoside, amentoflavone, phenolic acids such as gallic acid. All extracts exhibited high cellular antioxidant activity in the range of 0.1 to 10 $\mu g \cdot mL^{-1}$ using lucigenin with neutrophils, but a moderate cellular antioxidant activity ranging between 10 and 100 $\mu g \cdot mL^{-1}$ with DCFDA on HL60 monocytes. Extracts from *Manihot* leaves showed a pronounced inhibitory effect on the production of extracellular ROS, on HRP and myeloperoxidase activity. Cellular antioxidant activities, the inhibitory effect on HRP of extracts from *M. glaziovii*, *M. esculenta* cultivar *Mwambu* were significantly higher, but their inhibitory effect on the activity of MPO was lower than those of *M. esculenta* cultivar TEM 419. The biological activi-

ties of *Manihot esculenta* and *Manihot glaziovii* were well correlated to their phytochemicals that could justify their traditional use as vegetables, potential functional foods or nutraceutical resources and medicines.

Keywords

Horseradish Peroxidase, Kahemba, Konzo, *Manihot esculenta*, *Manihot glaziovii*, Myeloperoxidase, Traditional Vegetable

1. Introduction

Manihot esculenta, Crantz L. called Cassava, constitutes part of the staple diet for more than 600 million people across the world [1]. Cassava is an important food crop in the tropics, it is the third most important source of calories, after rice and maize according to the Food and Agriculture Organization (FAO) [1] [2]. Cassava leaves and roots are excellent sources of carbohydrates, vitamins and mineral elements, but the roots contain very little protein. All parts of the plant contain toxic compounds that are cyanogenic glycosides (linnamarin, lotaustralin). The consumption of Cassava needed the particular transformation process and better preparation of roots and leaves for eliminating cyanogenic glycosides. *Manihot esculenta* (Euphorbiaceae) leaves are currently consumed as vegetables by the people in the origin countries (Africa, Latin America and Asian) and by migrants from Sub-Saharan Africa living in Western Europe [3]. The Congolese population of the DRC is heavily dependent on Cassava, and Bell *et al.* (2000) reported that Cassava is "all good enough" for the Congolese people because they receive the bread of the roots and the meat of the leaves [4]. This dependence is high for the rural populations such as Kahemba's population. Kahemba is a rural area of the Kwango region in DRC, which is severely affected by konzo. Konzo is a neurological disease associated with chronic dietary reliance on foodstuffs from insufficiently processed bitter cassava [5]. Cassava cultivars (varieties) are classified as sweets and bitters cassava. Households of Kahemba cultivate some wild, ameliorate sweet and bitter wild varieties of Cassava and the bitter types predominate for their yields, more drought and infection, insect resistants. Among these varieties, the most preferred are *Mwambu*, *Tshibombi* and TEM 419 cultivars. *Mwambu and Tshibombi* are the bitter wild varieties of Cassava and TEM 419 is the sweet ameliorate variety introduced by FAO [6]. Cassava is the main staple food of Kahemba's population. Common foodstuffs from roots are essentially cassava bread-like items known as *chikwange*, *fufu*, stiff pastes made from cassava flour. This common staple food is largely consumed together with *saka-saka or pondu*, a sauce prepared from cassava leaves [7]. Besides the leaves of Cassava (*Manihot esculenta*), the leaves of *Manihot carthaginensis* subsp. *glaziovii* (Müll.Arg.) Allem (*Manihot glaziovii*) were equally consumed as a traditional vegetable (**Figure 1**).

Figure 1. Leaves of *Manihot esculenta* (Mwambu: A; TEM 419: B) and *Manihot glaziovii* (C) from Kahemba.

The leaves of *M. glaziovii* were only consumed in the west of the Democratic Republic of the Congo to the best of our knowledge [6]. Elsewhere *M. glaziovii* is particularly used as biomass or raw material for bioethanol and natural rubber production [8] [9]. The vegetable sauces constitute the main protein sources for the population of Kahemba, which does not consume meat and fish daily. Based on the very high consumption of *Manihot* species leaves as a vegetable in DRC, it is worth determining their potential bioactivities and nutritive values. Few reports had documented the bioactivities of the leaves of *M. esculenta* and *M. glaziovii*. Tsumbu *et al.* (2011, 2012) [3] [10] had evaluated the polyphenol content and modulatory activities of *M. esculenta*, some green vegetables from Kongo Central. The present paper reports the microscopic features, chromatographic fingerprints and the biological activities of leaves of edible *Manihot* species from DRC used such as traditional vegetables.

2. Materiel and Methods

2.1. Plant Material

The leaves of *Manihot esculenta* and *Manihot glaziovii* have been collected from the areas of Kahemba (DR. Congo) in April 2018. The identity of the plant material was established by Mr. Kombo (Agronomist at the Ministry of Agriculture/Kahemba), and was confirmed by Mr. Anthony Kikufi, biologist from the University of Kinshasa (DR. Congo). The leaves were soaked in hot water (100˚C: 1 to 3 minutes), drained and dried at room temperature. The reduction to powder of leaves was done by using a high-speed mill (Retsch ZM 100 Model).

2.2. Chemicals

All solvents used were of analytical and HPLC grade and purchased from Merck

VWR (Leuven, Belgium). 2-Aminoethyldiphenylborat and Phorbol-12-myristate-13-acetate (PMA) were purchased from Sigma (Bornem, Belgium). 2',7'-Dichlorofluorescein-diacetate (DCFH-DA) was purchased from Eastman Kodak (Rochester, NY, USA). L0-12 (8-amino-5-chloro-7-phenylpyrido [3,4-d] pyridazine-1,4(2H, 3H) dione) was purchased from Wako Chemicals Gmbh (Neuss, Germany). Horseradish Peroxidase (HRP) was obtained from Roche (Mannheim, Germany) and human Myeloperoxidase was from Calbiochem, EMD Millipore (Bellirica, MA USA). Gallic acid (purity: 97%) was purchased from Sigma-Aldrich. Rutin (purity ≥ 99%), isoquercitrin (purity ≥ 99%) and, Hyperoside (purity ≥ 98.5%) were HPLC grade and purchased from Extrasynthese. Water was treated using a Milli-Q water ultra-purification system before use.

2.3. Microscopic Analysis

Microscopic observations were made using lactic acid reagent [11]. Observations and pictures were done with a Zeiss Primo Star microscope coupled to camera (DP 200).

2.4. Preparation of Extracts

Methanolic extracts were prepared by percolation with methanol from 10 g of leaf powder to obtain 200 mL of percolate. The evaporation of the solvent was performed under reduced pressure (at 40˚C) followed by 48 - 72 h stay in a vacuum chamber. The extracts were then weighed and kept in dark hermetic flasks at 4˚C.

2.5. Chromatographic Analysis

Analytical analysis by Thin Layer Chromatographic of 10 µL of solution for 10 mg/mL of methanolic extracts was carried out on normal phase Silica Gel 60 F_{254} plates (Merck, Darmstadt, Germany), using a mixture of solvents as suitable eluents. The plates were visualized at 365 nm with Neu reagent [12].

The separation of phenolic compounds of methanolic extract was carried HPLC-DAD out on a Hypersil ODS® RP18 column as described previously [13].

2.6. Cellular and Enzymatic Assays

2.6.1. Cellular Antioxidant Activity

1) Cell culture and treatment

Human promyelocytic leukemia cells (HL-60) were obtained from the American Type Culture Collection (ATCC, the USA) and cultured in the appropriate medium (IMDM obtained from Biowest, France). Equine Neutrophils were isolated as described previously [10].

2) Measurement of Cellular Antioxidant Activity (CAA)

a) Measurement of the ROS produced by PMA-Activated HL-60 monocytes (fluorescence technique with non-fluorescent DCFH-DA)

This technic was based on the method described previously [13] [14].

b) Measurement of the total ROS produced by PMA activated neutrophils (chemiluminescence assay)

The ROS produced by activated neutrophils were measured by lucigenin-enhanced chemiluminescence (CL) as reported by Franck *et al.* (2013) [15].

2.6.2. Inhibition of Peroxidase Activity

1) Inhibition of myeloperoxidase activity

This test was carried out using the SIEFED method that evaluate the capacity of chemical compounds such as phytochemicals extracts from natural products to modulate the activity of MPO [15].

2) Inhibition of HRP oxidant activity

The used method evaluates the modulatory effect of chemical compounds or extracts from natural products on HRP catalytic activity using L012, a luminol-based chemiluminescent probe as described previously [16].

2.7. Cell Viability

Cell survivals rate were quantified using a classic colorimetric WST-1 assay to measure mitochondrial activity in viable cells [17] and an exclusion test with Trypan blue [18].

2.8. Statistical Analysis

The software was performed with GraphPad 7.0 (GraphPad Software, San Diego California, the USA) was used to perform statistical analysis. Two-way analysis (ANOVA), Student's paired t-test, "Tukey" Multiple Comparisons Test were the test performed. The level of statistical significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Botanical Microscopic Characteristics

Powders from the leaves of *Manihot* species showed the following specific botanical microscopic characters. *Manihot esculenta* (*Mwambu*) revealed the abundance of spherical starch granules ~3 - 18 µm diameter, large underlying palisade cells, calcium oxalate prism ~38 µm long, upper epidermis in surface view, showing thicker-walled cells, abundant lignified fibers, isolated sclereids up to ~53 × 32 µm (L × W), the group of isodiametric sclereids, fibrous sclereids up to ~335 × 20 µm (L × W) usually strung at one end, unicellular non glandular trichomes up to ~172 µm long, diacytic stomata, epidermis of polygonal cells, the fragment of bordered pitted vessels (**Appendix Figure 1S**). *M. esculenta* cultivar TEM 419 showed large underlying palisade cells and calcium oxalate prism ~27 × 15 µm (L × W), few starches granules up to 17 µm diameter, diacytic stomata, unicellular non-glandular trichomes ~146 - 218 µm long, scalariform vessels, lignified fibers, the group of sclereids, isolated elongated sclereids ~35 - 85 µm long, fibrous sclereids ~ up to 290 × 20 µm (L × W), diacytic stomata (**Appendix Figure 2S**). *M. glaziovii* revealed abundant lignified fibers, large underlying pa-

lisade cells ~30 - 11 μm (L × W), parenchyma with clusters crystal of calcium oxalate ~8 μm diameter, upper epidermis in surface view showing thicker-walled cells, few starches granules (~6 - 18 μm diameter), numerous smooth unicellular no glandular trichomes up to 277 μm long, glandular trichomes, cyclocytic stomata, the fragment of helical vessels, elongated sclereids ~92 × 36 μm (L × W), and fibrous sclereids up to ~248 × 18 μm (L × W) (**Appendix Figure 3S**). The stomata, vessels of *M. glaziovii* are different of those from *M. esculenta*. The number and dimension of the starches granules, sclereids, and fibrous sclereids are not also the same. Sclereids, starches granules from *M. esculenta* were usually small than those of *M. glaziovii.* Microscopy analysis allowing the identification of herbal drugs and the detection of individual components of a mixture [19], the obtained results could contribute to characterize *Manihot* species.

3.2. Phenolic Compounds

Chromatographic fingerprints of methanolic extracts hinted at the presence of flavonoids and phenolic acids as major phytochemicals. By comparison with used standards, it was showed that extracts of *M. esculenta* contain quercetin-3-rutinoside (rutin), the most abundant, amentoflavone, isoquercitrin, kaempferol-3-rutinoside and other non-identified flavonoids (**Figure 2** and **Figure 3**), in accordance with previous results [3] [20]. *M. glaziovii* contained equally amentoflavone, quercetin-3-rutinoside, kaempferol-3-rutinoside but also quercetin-3-glucoside (**Figure 4**).

Quercetin has been also identified in the two species. Caffeic acid, gallic acid and others non-identified acids were also detected in the two species. Ola *et al.* (2009) [20] reported that ferulic acid is the main phenolic acid from leaves of *M. esculenta* from Nigeria. In our study, there is not ferulic acid in Manihot extracts

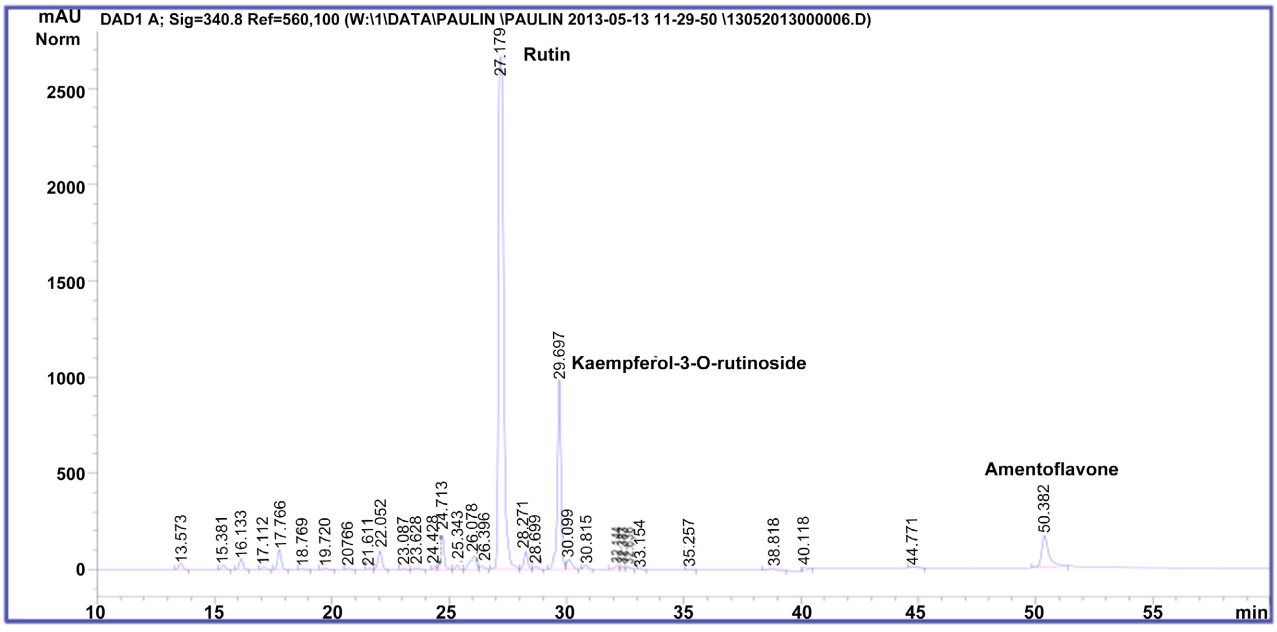

Figure 2. HPLC-DAD chromatogram of methanolic extract from *Manihot esculenta* (cultivar Mwambo).

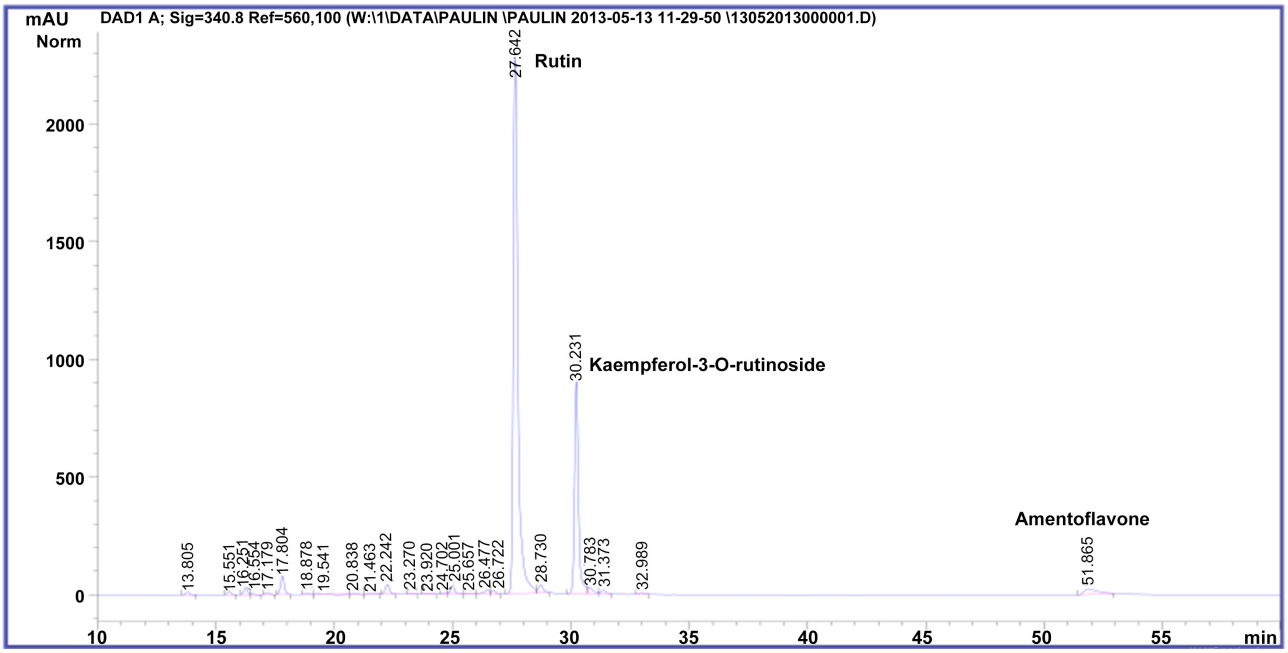

Figure 3. HPLC-DAD chromatogram of methanolic extract from *Manihot esculenta* (cultivar TEM 419).

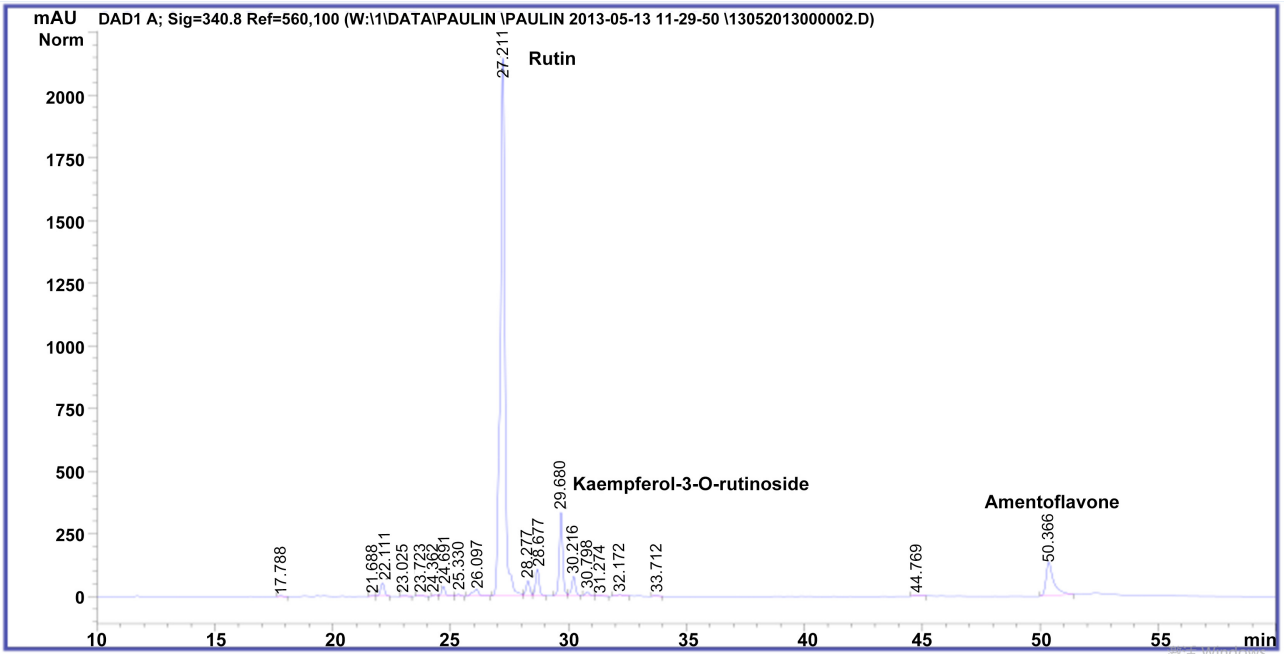

Figure 4. HPLC-DAD chromatogram of methanolic extract from *Manihot glaziovii*.

as shown the TLC and HPLC fingerprints in comparison with standard of ferulic acid. The chemical composition of plant extracts is related to different parameters such as varieties, genetic, ecology, harvest conditions and the types of extracts. Chromatographic fingerprints of samples from studied *Manihot* species were nearly similar (**Figures 2-4**). Nevertheless, chromatographic analysis revealed that the two variety of *M. esculenta* had a chemical fingerprint different from *M. glaziovii*.

3.3. Cellular Antioxidant Activity

Cell-free antioxidant assays were largely used to evaluate the antioxidant activity of pure compounds and plant extracts. Cellular models such as those using cells specialized in the production of reactive species and inflammatory responses allow the evaluation of antioxidant and anticatalytic capacities as a complement to cell-free antioxidant assays. In this study we evaluated the capacities of extracts to modulate the ROS production resulting mainly from NADPH oxidase activity by stimulated neutrophil and HL-60 cells [21].

On the one hand, in the range of the concentration of 0.1 to 10 μg·mL^{-1} for extracts from Manihot leaves and of 10^{-6} to 10^{-4} M for positive controls (gallic acid and quercetin), we had observed a significant decrease of the neutrophils ROS production compared to the control test performed with DMSO. Obtained results showed that the cellular antioxidant activity of extracts is significantly higher ($p < 0.001$) in the following order: *M. glaziovii* > *M. esculenta* (*Mwambu*) > *M. esculenta* (TEM 419) (**Figure 5**).

The highest inhibitory effect was related to their polyphenolic content and the obtained IC_{50} were 0.11 ± 0.05 μg·mL^{-1}, 0.14 ± 0.03 μg·mL^{-1} and 0.69 ± 0.15 μg·mL^{-1} for *M. glaziovii*, *M. esculenta* (*Mwambu*) and *M. esculenta* (TEM 419) respectively. The total phenol contents of *M. glaziovii*, *M. esculenta* (*cultivar Mwambu*) were two to three higher than *M. esculenta* (cultivar TEM 419), which correlated to their antiradical activity as recently reported [6].

On the other hand, at the concentration of 10, 50 and 100 μg·mL^{-1} the leaf

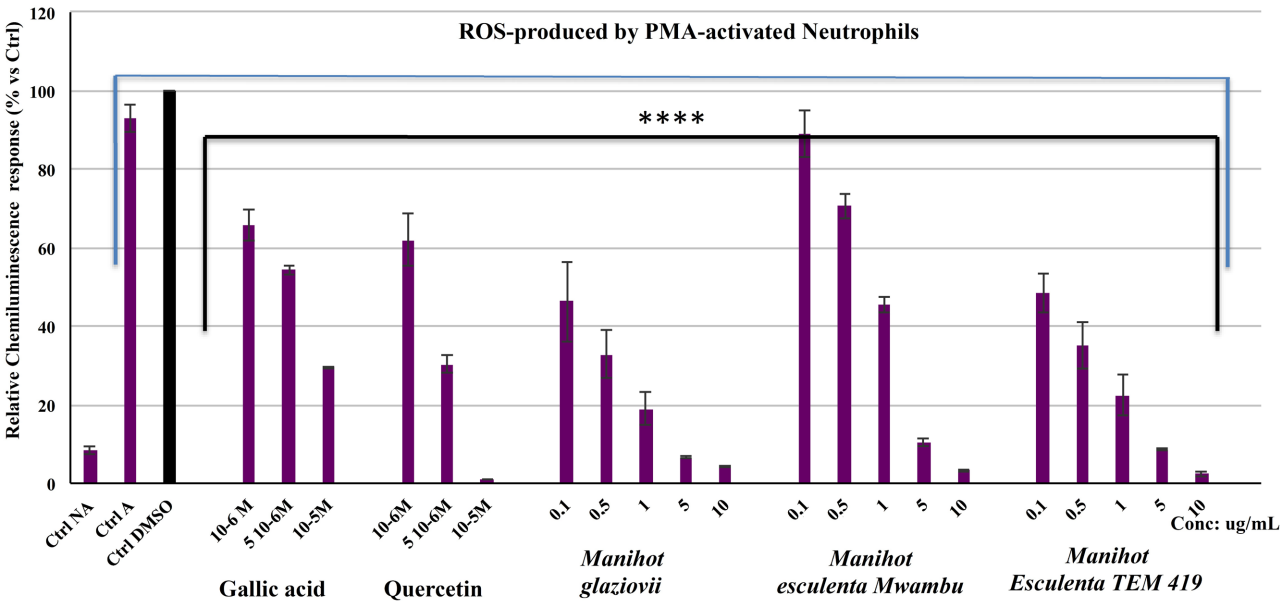

Figure 5. Effects of gallic acid, quercetin and methanolic extracts of Manihot species on the CL response produced by PMA activated equine neutrophils (Means ± SD, n = 6). The CL intensity results from the reaction between lucigenin and the ROS produced by the non-activated (NA) and activated equine neutrophils (A). The CL response of stimulated neutrophils in the presence of DMSO used to solubilize the extracts was defined as 100%. P-values (****$p < 0.0001$) calculated by two-way ANOVA followed by Sidak Multiple Comparisons Test indicated a significant effect of the extracts vs. DMSO control; ns = not significant vs. DMSO control.

Manihot extracts and of 10^{-6}, 10^{-5} 10^{-4} M the quercetin have produced a low dose-dependent decrease of HL-60 ROS production compared to the control test performed with DMSO. Obtained results with this cellular model showed that the effect of methanolic extracts is significantly higher ($p < 0.05$) only at the 100 $\mu g \cdot mL^{-1}$ in the following order: *M. glaziovii* > *M. esculenta* (*Mwambu*) > *M. esculenta* (TEM 419) compared to the previous model using lucigenin on neutrophils. At 100 $\mu g \cdot mL^{-1}$, the percentage of ROS inhibition was of 38.36%, 36.87% and 30.09% for *M. glaziovii*, *M. esculenta* (*Mwambu*), *M. esculenta* (TEM 419) respectively.

Indeed, *Manihot* extracts were active in the two cell-models assays, and showed a more pronounced inhibitor effects on ROS production in the lucigenin CL assay. Tsumbu *et al.* (2012) had reported the same observations with aqueous extracts of *Manihot esculenta* from another area of DRC, *i.e.* Kongo Central. Lucigenin is considered to be a more specific probe for the detection of superoxide anions directly produced by the activity of NADPH oxidase and released in the extracellular media [22]. DCFH-DA makes it possible to indirectly measure the effect of intracellular antioxidant activities against intracellular ROS production in fluorescence assay.

Regarding the results obtained in the fluorescence assay, we presumed that there would be interferences of ions and molecules of plant extracts on the modulatory effect on intracellular ROS production. For this, assays with EDTA and Chelex, both being used to complex metallic ions, were performed in the first hand for excluding the Fenton-like reaction. The reaction between H_2O_2 and Fe^{2+} (Fenton reaction) leads to the formation of hydroxyl radical (•OH) that can oxidize DCFH to DCF. The Fenton reaction might lead to DCF-amplified fluorescence that could lead to the low inhibitory effect on ROS production [23]. On the other hand, we tested the possible interferences between the probe (DCFH-DA) and compounds in the extracts. Assays were performed by comparing the fluorescence intensity of the cells (HL60 monocytes), incubated with the probe, and extracts compared to that obtained when HL60 were incubated with the extracts without the probe.

The obtained results suggested that there were not any ionic and molecular interference: This indicated that the components of the tested extracts were not very good intracellular ROS scavengers. Tested extracts contained glycosylated flavonoids as major phenolic compounds. Previous studies reported that methanolic extracts of edible *Hibiscus* and herbal teas from DRC exhibited high effect on intracellular ROS related to their abundance of phenolic acids [13] [24]. In DCFH-DA fluorescence assay, flavonoids seem to be less active than phenolic acids. Assays with molecules of flavonoids (quercetin and its glycosides) and phenolic acids standards in the range concentrations of 10^{-6} - 10^{-4} M, showed that phenolic acids were more active to inhibit intracellular ROS than flavonoids. For flavonoids, genins were more active than glycosides and the glycosides with one sugar are more active than those with several sugars.

Nevertheless, Takamatsu *et al.* (2003) [25] showed that the antioxidant efficacy in DCFH-DA fluorescence model depends on the nature of substituents of the rings of flavonoids and to a great extent on the ability of molecules to penetrate the cell membranes.

3.4. Inhibition of Peroxidase Activity

The evaluation of the inhibition of peroxidase activity carried out with tests using as enzymes the Myeloperoxidase (MPO) and the Horseradish Peroxidase (HRP).

MPO, a pro-oxidant enzyme involved in secondary cell damage and considered as a marker of inflammation [15]. In SIEFED (Specific Immunological Extraction Followed by Enzymatic Detection) technique, at the concentrations of 1, 5 and 10 $\mu g \cdot mL^{-1}$, all *Manihot* extracts showed a significant inhibitory effect ($p < 0.001$) on MPO activity in the following order: *M. esculenta* (TEM 419) > *M. esculenta* (*Mwambu*) > *M. glaziovii*. The observed effect showed that molecules of *Manihot* extracts interact better with the active site of MPO. The SIEFED technic is an immunological test which allows detecting compounds that have direct interaction with the MPO [15].

Altogether the results of our study showed that the extracts tested have the highest antioxidant activities and the highest inhibition on the activity of MPO. As reported by previous studies, molecules or the plant extracts which having a good antiradical or antioxidant activities are not necessarily good inhibitors of MPO activity. Gallic acid is less efficient MPO inhibitor compared to quercetin that is less antiradical [13] [25]. *M. glaziovii* has showed good antiradical and antioxidant activities than *M. esculenta* (TEM 419), but it had a low inhibitory effect on MPO activity. Phenolic acids and glycosylated flavonoids such as rutin were found to be the major phenolic compounds of *Manihot* extracts. Flavonoids were reported to be excellent inhibitors of MPO [26] [27]. Previous studies reported for benzoic acid derivatives, a pyrogallol and the elongation of the carboxylic group seem to be essential for the inhibition of MPO activity. These configurations would facilitate interactions of molecules with the MPO active site [28]. Gallic acid induced a more dose dependent anticatalytic activity on MPO than caffeic acid and its derivatives [13].

The inhibition of HRP oxidant activity was studied by chemiluminescence method using L-012 as probes. L0-12 is a chemical analog that has been reported to gives rise to significantly higher luminescence yield and increased sensitivity compared to other CL probes, such lucigenin [29]. HRP was used for the investigation of inhibitor activity of anti-thyroid and anti-inflammatory drugs [30].

At the concentration of 0.1; 1 and 10 $\mu g \cdot mL^{-1}$, *Manihot* extracts showed an effective inhibition of HRP oxidant activity (**Appendix Figure 4S**).

At the concentration of 10 $\mu g \cdot mL^{-1}$, the percentage of the inhibition effect was more than 50% for *M. glaziovii* and *M. esculenta* (*Mwambu*); and of 32.53% for *M. esculenta* (TEM 419). Regarding to our results with this assay, *Manihot* ex-

tracts exhibited a great capacity to inhibit HRP catalytic activity related to their phytochemicals. Flavonoids such as quercetin derivatives contained in *Manihot* extracts, could be responsible of the inhibition effect on HRP catalytic activity. Mahfoudi *et al.* (2017) [30] reported that flavonoids could be promising HRP inhibitors and can help in developing new molecules to control thyroid diseases.

The uncontrolled stimulation of neutrophils leading to neutrophil degranulation associated with some acute and chronic diseases, could contribute to amplify or maintain the inflammatory response with the release of peroxidases such as MPO [31]. The activity of MPO produces highly diffusible reactive oxidants, which provoke oxidative damage in the host tissues at inflammatory sites. MPO and its metabolites are as promising biomarkers not only for infectious diseases, but also for a wide array of non-infectious and neurodegenerative disorders [32]. Our results demonstrated that all tested extracts exerted a noticeable inhibitory effect on the MPO and on HRP catalytic activity. Polyphenols have by their antioxidant, anti-inflammatory capacities, may to confer health benefit in diverse neurodegenerative disorders associated with oxidative damage [33]. The inhibitors of HRP and MPO activity are promising therapeutic agents such as anti-inflammatory drugs.

Manihot species contain cyanogenic glycosides (α-hydroxynitrile glucosides) and leaves have high levels than roots. Cyanogenic glycosides (linnamarin, lotaustralin) break down to release toxic cyanide (HCN) when plant tissue is crushed or chewed, disrupting the cells [1]. The consumption of leaves such as vegetable needed a better culinary preparation. The processing preparation of *Saka-saka*, the sauce from cassava leaves has several stapes: blanching in warm water for a few minutes, grinding before pounding, boiling 30 minutes before the mixing with ingredients. The heat treatment and the consistency of pounding play a role in the reduction of cyanogens. Destruction of the cells leads to contact between the cyanogenic glucosides and the endogenous linamarase with the subsequent release of HCN. Ngudi *et al.* (2003) [34] reported that 96% - 99% of the total cyanogens were removed after cooking of the cassava leaves. Tested samples of *Manihot* species were submitted to heat pretreatment. We estimated that this treatment allowed the removing of the maximum of cyanogenic compounds before analysis and it does not affected the bioactivities of *Manihot* extracts on cellular and enzymatic models, which are essentially related to phenolic compounds and not to the toxic effect of cyanogens. For this, we performed the cell viability tests on HL-60 monocytes and neutrophils. The cell viability of HL-60 cells and equine neutrophils treated with *Manihot* extracts was significantly superior to the control group except at the highest concentration tested (10 $\mu g \cdot mL^{-1}$). The extracts of *Manihot* species caused no cell toxicity and there was no significant difference of viability between cells incubated with plant extracts and those without extract solutions (control cells). These results suggest that *Manihot* extracts do not have a toxic effect at the high concentration (10 $\mu g \cdot mL^{-1}$) and even have a slight protective effect against cell death at low concentrations (0.5 $\mu g \cdot mL^{-1}$). The *Manihot* ex-

tracts did not induce cytotoxicity at doses showing antioxidant and peroxidase inhibition activities.

Cassava production is growing in the peri-urban areas of Kinshasa for the exploitation of the roots as raw material for many processing products like liquid starch, cosettes, *chikwange* ... by local entrepreneurs. Cassava leaves are for the Congolese population, great nutritional and economic values as a source of proteins, minerals, and as a source of income for households. The nutritional value of this vegetable makes its consumption and marketing become more and more important in the DRC than abroad.

The cellular antioxidant and the inhibition of peroxidases activities of *Manihot* leaves were positively correlated with their phytochemicals. These bioactivities justify the benefit effect of *Manihot* leaves such as traditional vegetable and potential nutraceutical resources and medicines with beneficial health for Congolese people.

4. Conclusion

Microscopic features, chromatographic fingerprints and biological activities of two Congolese *Manihot* species were determined. The metabolic profile of *M. glaziovii* appears quite similar profiles to those of *M. esculenta*. Methanolic extracts tested have the best antioxidant activities. They appeared less efficient on the inhibition of the production intracellular ROS of HL60 cells, and more efficient as radical scavengers, on the inhibition of the production of extracellular ROS of neutrophils and the inhibition of MPO and HRP oxidant activities. The antioxidant and the inhibition of MPO and HRP activities of the leaves of the studied *Manihot* species would potential therapeutic interest and could justify their traditional use as vegetables, potential functional foods or nutraceutical resources and medicines. However, we estimate that further studies are needed, especially *in vivo* studies, to demonstrate the benefit of Manihot leaves extracts in health.

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Conflicts of Interest

The authors declare no conflict of interest.

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Appendix

https://drive.google.com/file/d/1K8GUu2Z2S1Rbit5tpos7OD0Ie-veJtmD/view?ts=6122d15f