Traditional Foods as Putative Sources of Antioxidants with Health Benefits in Konzo

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Additional information is available at the end of the chapter

Abstract

Konzo is a toxico-nutritional neurological disease associated with oxidative damage induced by cyanide poisoning through the ingestion of poorly processed bitter cassava. Dietary uses and patterns, determined using food frequency questionnaires, structured interviews and direct observation in consenting households to Kahemba, the rural area most affected by konzo in the world, showed that the diet of affected population is not varied and largely dependent on cassava (*Manihot esculenta* Crantz) products. Commonly consumed foodstuffs include herbal teas, mushrooms, spices, vegetables and yams. Phytochemical composition of extracts revealed that they contained flavonoids and phenolic acids as major compounds. All extracts of investigated traditional foods at the concentration range of 0.25–20 µg/mL, displayed high radical scavenging and cellular antioxidant activities using lucigenin on equine neutrophils, related to their phenolic content. The leaves of *Manihot esculenta* and *Manihot glaziovii* exhibited the highest antioxidant activity among vegetables. *Lippia multiflora* is the most active of the herbal teas, *Auricularia delicata* of mushrooms, *Dioscorea alata* of yams and *Ocimum basilicum* of spices. Traditional foods showed more efficient effects on extracellular ROS production and MPO activity. Traditional foods have interesting antioxidant, anti-inflammatory properties and could putatively be used as functional foods or nutraceuticals in the prevention of oxidative damage associated with konzo.

Keywords: anti-inflammatory activity, brain damage, cassava, cyanide, functional foods, nutraceuticals, oxidative stress

1. Introduction

Konzo is a permanent and non-progressive paralytic disease that affects thousands of children and women of child-bearing age among millions of people who rely on cassava as their main source of food, mainly in Sub-Saharan Africa [1]. We recently showed that cognition may also be affected and subtle or pre-clinical forms of the paralytic disease may exist. Thus, the global burden of the disease may therefore have been underestimated, which raises serious concerns for the public health of millions of people for whom cassava is the main subsistence crop [2, 3].

The mechanisms underlying konzo remain unclear, although epidemiological studies have consistently shown an association between the occurrence of konzo and chronic dietary reliance on foodstuffs from insufficiently processed bitter cassava, with poor protein intake. Our recent studies suggest that disease development may be mediated through oxidative damage, findings that appear to be consistent with the putative effects of cyanide intoxication and/or chronic undernutrition [4]. Konzo is a permanent and irreversible condition with no treatment available. Improved processing methods to remove cyanogens from cassava prior to human consumption and enhancement of human cyanide detoxification capabilities perhaps through dietary supplementation may be critical to the prevention of the disease [5]. However, because of the chronic and heavy dietary reliance on bitter cassava as the main food source and the potential for continuous exposure to residual amounts of cyanogens due to variations in processing methods, chronic low-dose exposure to cyanide may persist, which may, possibly, lead to oxidative damage and neurocognitive deficits. It is therefore important that preventive measures are embedded in daily food practices and dietary habits to avoid the unnecessary burden of toxicity related to cyanide. The promotion of traditional or ethnic foods with potential health benefits may be useful in konzo-affected areas.

In this chapter, we report findings from a survey of food consumption and a subsequent phytochemical composition of relevant foods, aiming to identify foods with interesting antioxidant properties that could be used as functional foods or nutraceuticals in the prevention of chronic cassava cyanogenic poisoning, including konzo.

2. Konzo

Konzo is a distinct neurological entity with selective upper motor neuron damage, characterized by an abrupt onset of an irreversible, non-progressive, and symmetrical spastic para/tetraparesis. The first description of the disease was done by the Italian doctor Trolli eight decades ago in the Democratic Republic of Congo (DRC), epidemics have been reported from many cassava-consuming areas in rural Africa such as Angola, Cameroon, Mozambique, Tanzania, the Central African Republic, the DRC and recently in Zambia [1, 2, 6]. The common feature of these affected areas is that Cassava (**Figure 1**) is the staple food associated with food and social insecurity, poverty and malnutrition. Cassava (*Manihot esculenta*) forms part of the staple diet for more than 600 million people across the world. The plant grows in poor soil and is relatively drought resistant. Cassava roots and leaves are a good source of carbohydrates

Figure 1. Leaves and roots of cassava (*Manihot esculenta*).

and some minerals and vitamins (vitamin C). The roots are a poor source of lipids and proteins. Manihot leaves contained anthocyanins, flavonoids and other polyphenols. All parts of the plant contained the cyanogenic glycosides (linamarin and lotaustralin) that constitute the antinutrient factors [7, 8].

In DR Congo, the dependence on cassava is particularly strong and it is estimated that cassava (*Manihot esculenta*) is "all good enough" for the Congolese people because they receive "the bread of the roots and the meat of the leaves [7].

Cassava contains cyanogenic glucosides (linamarin and lotaustralin) that are released as hydrogen cyanide, which are thought to protect the plants from insects and other animals. For human consumption, the plants need to be detoxified, usually by soaking, drying in the sun, boiling, fermentation, or grating with roasting. These processes allow the cyanogenic glucosides to be released, but depend upon traditional practices, time taken, and the availability of water. Major food crises following drought or war are the cause of konzo. In these situations, the traditional systems of processing cassava roots into flour and other derived products are completely modified by: (i) The reduction of cassava retting time that is achieved not in the river as practiced in traditional methods but within households in closed containers (ii) Reducing the drying time of roasted cassava, …(iii) Drying of cosettes or roasted cassava under a wood fire [8]. These changes in cassava root transformation expose the population to cyanide intoxication through the consumption of flour and other by-products with cyanide levels that exceed the WHO standards (maximum 10 parts per million: ppm). A 2011 survey of 123 households in Kahemba showed that the average cyanide level in cassava flour was 92.2 ± 56.2 ppm [9]. Neurotoxicity is associated with incompletely detoxified cassava, although the exact mechanisms by which these compounds cause neurological damage is unclear. Two neurological conditions are mainly associated with bitter cassava: a myeloneuropathy and konzo. The myeloneuropathy called tropical ataxic neuropathy (TAN) manifests as a slowly evolving bilateral sensory polyneuropathy, optic atrophy and sensorineural deafness, and sensory ataxia, is seen in adults (particularly elderly) who have a solely cassava diet. The toxicity of cyanide is reduced by its transformation to thiocyanate or cyanate, which requires sulfur donors, often limited in malnutrition. However, it has been shown that oxidative damage plays a crucial role in the pathogenesis of konzo [10].

Konzo is defined by World Health Organization (WHO), as a visible symmetric spastic abnormality of gait while walking or running in a formerly healthy person with a history of onset of less than 1 week. After onset, a non-progressive course follows and bilaterally exaggerated knee or ankle jerks without signs of disease of the spine. WHO definition dedicated konzo as a pure upper-motor neuron disorder, cognitive effects were originally deemed absent or minimal [11]. Recently, Boivin et al. showed that motor proficiency is dramatically affected, and both children with and without konzo have impaired neurocognition compared with control children from a no outbreak area. Therefore, konzo is associated with a subclinical neurocognitive form, extending the human burden of konzo with dramatic public health implications [2]. Dietetic macronutrients and micronutrients play a crucial role in the control of brain physiology, and food intake is known to stimulate the activity of neurotrophic factors regulating synaptic plasticity. In recent years, epidemiological studies have shown that the regular consumption of fruits, vegetables, and spices… had a lower incidence of cardiovascular, neurological disorders and others. Functional foods and nutraceuticals have been proven beneficial for the prevention or amelioration of cognitive impairments in degenerative diseases [12].

3. Traditional foods in diet of Kahemba's population

Kahemba in province of Kwango, has a special significance due to recurring outbreaks of konzo disease over the past 20 years and is severely affected by konzo [2]. Food frequency questionnaires (FFQ) were used to identify the most common food items consumed by the local population. The Ministry of Health for the Congo for the ethical conduct of human participant research provided study approval (MD/125/2013). Interviews were conducted with 30 consenting households (families) of which 13 had at least one child affected by konzo (konzo households). Direct observations were also made to document dietary habits adopted by the aforementioned families.

Dietary habits of Kahemba's population are centered on cassava, the main staple food. Common foodstuffs include cassava bread-like items known as *chikwange* and *fufu*, a stiff paste made from cassava flour (**Figure 2**). There are different processing techniques to detoxify cassava. These include soaking (retting) for 4 days minimum in water and drying in the sun outside or inside the house under firewood. In practice, the retting of cassava tubers is done in closed containers with little water. Sun drying usually takes several days, except certain households in konzo areas have reduced processing times mainly due to famine (**Figure 2**).

The number of meals per day varies between 1 and 3 depending on household income. These meals do not necessarily correspond to breakfast, lunch and dinner. For konzo or non-konzo households, the basic meal (cassava flour paste + condiment) is consumed 2–3 times and sometimes the condiment varies. Variability of meals per day is weak especially for konzo households due to lack of financial resources. In the majority of cases the same dish is split and eaten morning, midday and evening; consequently the food is not varied in spite of the availability of various traditional foods. For many households, the breakfast meal was cassava porridge, often cooked the day before.

Figure 2. Cassava processing and diet for children of Kahemba. Sun drying of cassava roots soaked in a container by a konzo household of Kahemba city (A) and the structure of a meal for households characterized by a high quantity of *fufu* accompanied by few vegetables and insects (B).

Few households consumed tea, coffee or herbal teas. Other households consumed boiled sweet cassava roots accompanied by peanuts, voandzous (*Vigna subterranea*) and avocado fruits. Konzo households mostly had one meal per day, consumed preferably in the evenings.

Readily available ethnic food items in the city of Kahemba, other than starch sources, included wild edible mushrooms, herbal teas, spices, vegetables (legumes), and yams. Among the mushrooms, *Auricularia delicata, Lactarius edulis, Lactarius symoensi* and *Schizophyllum commune* were the most abundant and readily available on the market all year round in dried form. For vegetables, leaves of *Manihot esculenta, Manihot glaziovii, Hibiscus cannabinus, Hibiscus sabdar-iffa, Ipomea batatas* and *Cucurbita maxima* are the most consumed by the two types of house-hold. Households cultivate some sweet and bitter local varieties of cassava roots. Mwambu variety is the bitterest variety of cassava and was introduced to Kahemba in the year 1937 from Angola. This variety is the most cultivated because its yield of tubers is the highest after 6 months [8].

Leaves of *Abelmochus esculentus, Abelmoshus moshatus, Amaranthus viridis, Gnetum africanum, Pteridium acquilium, Psophocarpus scandens, Sesamum angustifolium, Solanum gilo* and *Solanum aethiopicum* (fruit) are largely consumed by non-konzo households.

Fruit consumption by households was low despite the variety of fruits cultivated by house-holds in their family plots. Fruit production was rather intended for sale and not consumption by the members of the households.

Caterpillars, larvae, and red meat, especially pig meat and fish, are largely consumed by the households but in small quantities.

Our findings indicate that konzo households mostly rely on cassava derived products as their main source of food and have limited access to other types of food including vegetables, fish and meats. These findings are consistent with previous studies that suggest that poor nutri-tion is a risk factor for konzo. This dietary pattern based principally on cassava flour paste exposes the consumer to intoxication with cyanide, especially in children for whom the quan-

tity of cassava flour paste represents more than 90% of the consumed meals. Previous studies showed that this dietary pattern is responsible for the persistence of konzo [9]. The Flora of province of Kwango is rich in traditional foods that are mostly unexploited. For this reason, Mbemba et al. assessed the nutritional value of some traditional foods by determining their relative amino acid composition, in order to contribute to the equilibration of the diet in the population of this area severely affected by malnutrition and konzo. Yams such as *D. alata* and *D. cayenensis* are the most widespread. Mbemba et al. reported that some dried yams are richer in proteins than cassava root and can be used with success to prepare porridge for children who represent the most at-risk population for konzo [13]. They are an alternative substitute for cassava especially as they are abundant during the dry season, a period corresponding to high outbreaks of konzo. Among the sources of animal proteins consumed, the fish *Chanmellabes tupus* (known locally as Misombi) and larvae are the foods economically accessible for the majority of Kahemba's population.

4. Traditional foods as functional foods and nutraceuticals

Functional foods are in fact products that may look like or are a conventional food and be consumed as part of a usual diet, but apart from supplying nutrients they can reduce the risk of chronic diseases. Nutraceuticals are health promoting compounds or products that have been isolated or purified from food sources and they are generally sold in a medicinal (usually pill) form [14, 15]. Considering these definitions, we noticed that these products contain bioactive compounds called phytochemicals that are capable of modulating metabolic processes and resulting in the promotion of better health. By having antioxidant, anti-inflammatory, immunomodulatory, adaptogenic, anticancer, and several other health benefits, functional foods and nutraceuticals are used worldwide for the prevention and treatment of chronic diseases such as diabetes, arthritis, cardiovascular and respiratory disorders, neurodegenerative diseases, and cancer [12].

4.1. Phytochemicals

Phytochemicals are found in fruits, herbal teas, mushrooms, spices, vegetables and whole grains. They include an extremely heterogeneous class of compounds (polyphenolic compounds, carotenoids, tocopherols, phytosterols, and organo-sulfur compounds) with different chemical structures (hydrophilic or lipophilic).

TLC and HPLC-DAD analysis of methanolic extracts of the most consumed foodstuffs of Kahemba, have shown the presence of polyphenolic compounds in all extracts. TLC fingerprints of extracts showed the presence of glycosylated flavonoids (yellow, orange and green fluorescent spots) and phenolic acids (blue fluorescent spots) as major compounds especially for vegetables and herbal teas (**Figure 3**). Cassava flours, mushrooms and yams contain mainly phenolic acids. Flavonoids identified were derivatives of quercetin (rutin, hyperoside, isoquercitrin, quercitrin) and kaempferol (Kaempferol 3-O-glucoside, kaempferol 3-O-rutinoside). Phenolic acids were caffeic acid and its derivatives such chlorogenic acid ferulic acid and verbascoside.

Figure 3. TLC chromatogram of methanolic extracts from *M.esculenta* (varieties: *Chamusuku, Disanka, Kamonji, Mwambu, Tshibombi, TEM 419*), *M. glaziovii* with astragalin (ast), caffeic acid (caf), chlorogenic acid (chl), ferulic acid (fer), luteolin(lut), quercitrin(que) and rutin (rut) as standards; developed with ethyl acetate/formic acid/methanol/water (20:0.5:2.5:2; v/v/v/v) and visualized at 365 nm with natural products-PEG reagent. Flavonoids are detected as yellow-orange fluorescent spots and phenolic acids as blue fluorescent spots.

Leaves of *Manihot esculenta* and *Manihot glaziovii* contained amentoflavone, quercetin, quercetin-3-rutinoside, quercetin-3-glucoside, and kaempferol 3-rutinoside as flavonoids and caffeic acid, ferulic acid, gallic acid as phenolic acids. Cassava roots, cossettes and cassava flours contained phenolic acids, such as ferulic acid, as major compounds [8]. *Hibiscus acetosella* contained 2-O-trans-caffeoyl-hydroxycitric acid as major phenolic compounds and flavonoids such as quercetin-3-galactoside. *Hibiscus cannabinus and Hibiscus sabdariffa* contained neochlorogenic acid as major phenolic compounds and flavonoids such as quercetin-3-rutinoside, quercetin-3-glucoside and kaempferol 3-rutinoside (**Figure 4**) [16].
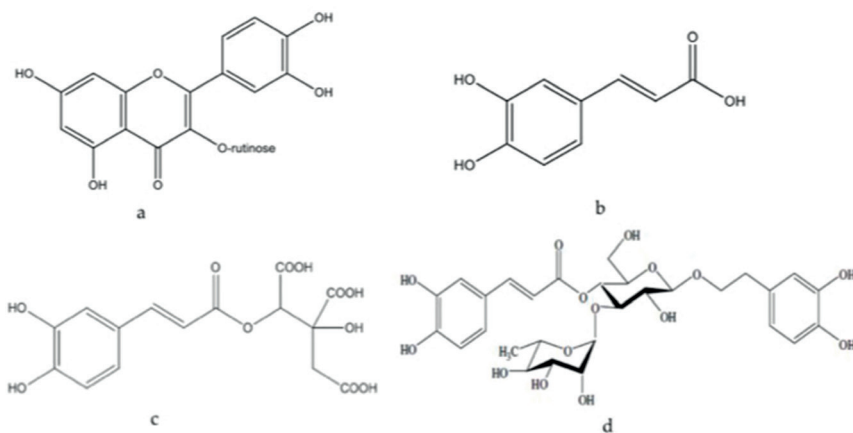

Figure 4. Structures of phytochemicals (a) quercetin-3-O-rutinoside, (b) caffeic acid, (c) Caffeoyl-hydroxycitrique, and (d) verbascoside.

Verbascoside is found to be abundant in herbal teas [17] and we first report here its presence in leaves of *Sesamum angustifolium*. Chlorogenic acid is the main phenolic acid found in extracts of *Raphia sese, Solanum aethiopicum* and *Solanum gilo*. Rosmarinic acid was found to be a major compound of Ocimum species of DRC [18]. Phytochemical screening of mushrooms and yams showed that phenolic acids are their major compounds. Phytochemicals derived from various sources target inflammatory and oxidative stress pathways and retard or delay the onset of neurological diseases.

Protective effects of phenolic compounds have often been ascribed to their direct antioxidant effect and/or to their anti-inflammatory action [12].

4.2. Antioxidant properties

Oxidative stress is recognized as an important factor in a variety of neurodegenerative diseases, as a mediator of the adverse effects of a number of neurotoxicants, and as a mechanism for age related degenerative processes [12]. Finding alternative and complementary ways to reduce the redox processes might have a beneficial interest in the context of developing countries.

Traditional foods contained a considerable amount of phenolic compounds expressed as total polyphenol contents as described in **Tables 1–3**. Total polyphenol contents of Kahemba's ethnic foods varied significantly between the samples of each group of foods. *Lippia multiflora* had the highest total polyphenol content among herb-teas, *Manihot glaziovii* among vegetables, *Cantharellus rufopunctatus* among mushrooms and *Dioscorea alata* among yams. Antioxidant activities significantly varied also between the samples of each group of foods ($p < 0.05$). All methanolic extracts had significant radical scavenging effects with increasing concentrations in the range of 1–40 μg/mL for herb teas, 10–80 μg/mL for spices and vegetables, and 10–250 μg/mL for mushrooms. This antiradical activity is connected to their ability to scavenge free radicals according to their IC_{50} values (**Tables 1–3**). IC_{50} values ranged from 7.56 ± 0.87 μg/mL (*L. multiflora*) to 653.13 ± 51.25 μg/mL (*D. cayenensis*) for the ABTS assay and from 10.44 ± 1.13 μg/mL (*L. multiflora*) to 17179.09 ± 1150.25 μg/mL (*Cantharellus* sp.) for the DPPH assay. Cassava flours also exhibited a good scavenging activity with IC_{50} values ranging from 99.54 ± 9.60 to 974.99 ± 94.01 μg/mL.

Beside conventional cell-free antioxidant assays, it can be pertinent to evaluate the anti-oxidant and anti-catalytic potential of plant extracts in cellular models involved in ROS production and inflammatory responses [16, 19]. The addition of extract solutions at increasing concentrations resulted in a dose dependent decrease of the ROS-induced lucigenin-amplified chemiluminescence. All tested extracts induced a significant inhibition ($p < 0.0001$) of the ROS production by neutrophils compared to controls at the concentration range of 0.05–10 μg/mL for herbal teas and vegetables; of 5–20 μg/mL for mushrooms (**Figure 5**).

Aqueous and methanolic extracts of Herbal teas showed also the best cellular antioxidant activity using DCFH-DA on HL-60 monocytes assay at 1–20 μg/mL [17]. The lucigenin-dependent chemiluminescence (CL) and the intracellular fluorescent probe DCFH-DA were used to evaluate the extra- and intracellular ROS production resulting mainly from NADPH oxidase activity by stimulated neutrophil and HL-60 cells [16].

Vegetables	TPC	AOX IC$_{50}$ (µg/mL)	
		ABTS	DPPH
Abelmoschus esculentus Linn	32.94 ± 0.93	86.27 ± 9.2	nd
Abelmoschus moshatus Medik	36.32 ± 1.05	52.36 ± 2.1	71.45 ± 14.44
Amaranthus viridis L.	23.31 ± 0.92	88.9 ± 11.1	762.08 ± 155.34
Dioscorea praehensilis	34.09 ± 4.46	106.44 ± 17.36	230.14 ± 31.07
Hibiscus cannabinus L.	89.05 ± 11.92	44.98 ± 0.87	73.79 ± 17.20
Hibiscus sabdarifa L.	82.97 ± 3.27	64.72 ± 6.17	86.04 ± 4.32
Ipomea batatas L.	76.78 ± 3.20	47.76 ± 3.25	233.35 ± 63.53
Manihot esculenta Crantz var. Chamusuku	41.51 ± 0.26	23.28 ± 1.11	nd
Manihot esculenta Crantz var. Kamonji	45.18 ± 0.79	17.65 ± 1.13	nd
Manihot esculenta Crantz var. Mwambo	86.4 ± 2.99	15.10 ± 1.13	20.15 ± 1.07
Manihot esculenta Crantz var. Tshibombi	57.56 ± 6.19	19.45 ± 1.11	40.93 ± 1.91
Manihot esculenta Crantz var. TEM 419	36.70 ± 4.16	23.07 ± 1.11	37.93 ± 2.25
Manihot glaziovii Mûll. Arg	107.71 ± 7.80	12.42 ± 2.08	20.5 ± 1.06
Megaphrynium macrostachum	32.69 ± 3.65	79.25 ± 10.29	503.5 ± 10.29
Sesamum angustifolium auct[*]	63.76 ± 3.76	31.19 ± 1.07	48.3 ± 1.02
Solanum aethiopicum L	32.24 ± 4.13	123.89 ± 16.15	282.49 ± 27.81
Solanum gilo Raddi (leaves)	72.04 ± 1.70	29.51 ± 0.94	163.68 ± 30.41
Solanum gilo Raddi (fruits)	24.19 ± 0.37	81.97 ± 5.17	349.95 ± 19.03

[*]*Sesamum angustifolium*, one of banned vegetables for konzo households by local traditional medicine [2], is the most active (antioxidant activity) among the vegetables after *Manihot* species. nd = not determined.

Table 1. Total phenolic content (TPC) of vegetables, expressed in mg of gallic acid equivalent (GAE) per g of dried matter and IC$_{50}$ (µg/mL) values of organic extracts in ABTS and DPPH assays (means ± SD, n = 6).

For the antioxidant activity, *L. multiflora* is the most active for herbal teas, *M. glaziovii* for vegetables and *A. delicata* for mushrooms related to their hydrophilic and lipophilic compounds [20]. The vegetable *D. praehensilis* exhibited higher cellular antioxidant activity than *I. batatas* and *S. gilo* (leaves) whereas these showed a superior activity to *D. praehensilis* for radical scavenging activity. López-Alarcón and Denicola showed that a good antioxidant is not just a good radical scavenger [21].

According to the scientific data, our study is the first to evaluate the antioxidant capacity of local traditional foods in an area severely affected by konzo disease in order to establish scientific basis of their use in the prevention of chronic cassava cyanogenic poisoning.

Eight species of edible mushrooms used in this study showed an interesting antioxidant activity compared to results reported in previous studies [22, 23]. There are few reports regarding the antioxidant activities of the studied mushrooms. *A. delicata* exhibited the highest antioxidant activity in comparison to other vegetables. However, Kabuyi et al. (2017) assessed

Mushrooms	TPC	AOX IC$_{50}$ (µg/mL)	
		ABTS	DPPH
Amanita loosii	8.82 ± 0.01	45.65 ± 1.00	1862.1 ± 425
Auricularia delicata	9.53 ± 0.12	39.31 ± 1.04	252.4 ± 15.5
Cantharellus sp.	4.73 ± 0.02	220.3 ± 17.40	1717.09 ± 522
Cantharellus symoensii	6.4 ± 0.02	144.9 ± 21.80	1367.73 ± 364
Cantharellus rufopunctatus	10.32 ± 1.09	41.1 ± 1.02	1815.52 ± 418
Lactarius tenellus	5.96 ± 1.47	43.51 ± 1.04	1603.25 ± 294
Lactifluus edulis	5.12 ± 0.11	262.4 ± 20.74	1318.3 ± 259
Schizophyllum commune	9.77 ± 0.40	169.8 ± 23.80	307.61 ± 25.05

Mushrooms exhibited a relatively interesting activity similar to some vegetables despite their low total phenol content.

Table 2. Total phenolic content (TPC) of mushrooms, expressed in mg of gallic acid equivalent (GAE) per g of dried matter and IC$_{50}$ (µg/ mL) values of organic extracts on ABTS and DPPH assays (means ± SD, n = 6).

Herb-teas	TPC	AOX IC$_{50}$ (µg/mL)	
		ABTS	DPPH
Lantana montevidensis (Spreng.)	87.74 ± 1.66	21.11 ± 1.68	27.15 ± 3.50
Lippia multiflora Moldenke	110.35 ± 3.89	7.56 ± 0.87	10.44 ± 1.13
Ocimum gratissimum L.	71.88 ± 1.16	12.07 ± 0.84	21.76 ± 2.92
Spices			
Aeollanthus suaveolens	26.30 ± 3.72	41.82 ± 3.99	nd
Ocimum basilicum	6.52 ± 0.18	38.37 ± 3.13	136.77 ± 15.64
Raphia sese De Wild	10.08 ± 0.51	40.71 ± 1.05	518.8 ± 95.16

nd = not determined. Herbal teas exhibited considerable antioxidant activity.

Table 3. Total phenolic content (TPC) of herb-teas and spices, expressed in mg of gallic acid equivalent (GAE) per g of dried matter and IC$_{50}$ (µg/mL) values of organic extracts in ABTS and DPPH assays (means ± SD, n = 6).

the selenium content and the antioxidant capacity of wild edible mushrooms from Kenge, another rural area of Kwango (DRC) with a high prevalence of malnutrition. They reported that *Auricularia delicata, Lentinus cf. cladopus, Pleurotus tuberregium, Marasmius buzungolo* and *Schizophillum commune* showed the interest antioxidant activity and moderate quantity of selenium, and *L. cf. cladodus* had the highest concentration [24]. The radical scavenging activity of mushrooms found by ABTS assay is significantly higher than that obtained with the DPPH assay. This great difference could be explained by synergistic effects of mushroom hydrophilic and lipophilic compounds on the ABTS$^{•+}$ chromogen [16].

Tested vegetables showed high antioxidant activity and leaves of *Manihot* species exhibited a strong radical scavenging capacity. A significant difference was found between antioxidant
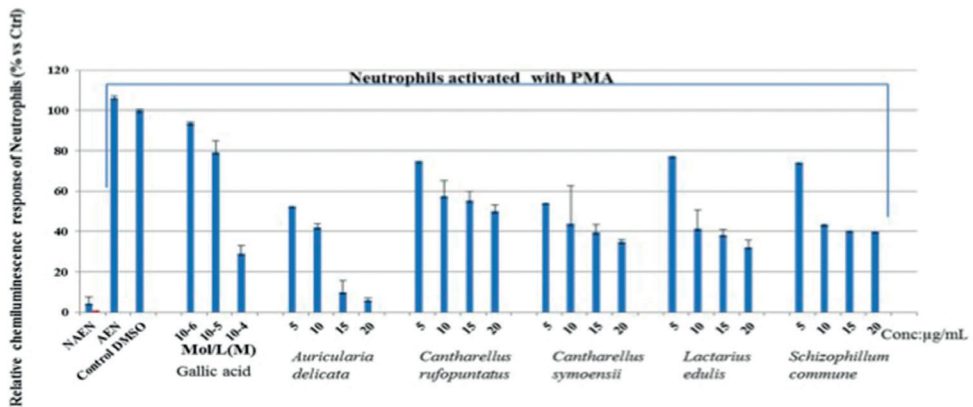
Figure 5. Effects of gallic acid and organic extracts of mushrooms on the CL response produced by PMA activated equine neutrophils (means ± SD, n = 6). The intensity of CL results from the reaction between lucigenin and the ROS produced by the non-activated (NAEN) and activated equine neutrophils (AEN). The chemiluminescence response observed in the presence of solvent (DMSO), used to solubilize the extracts, and was defined as 100%. For non-activated (NAEN) cells, 10 µL of PBS was added to the cell suspension instead of PMA.

activities of different varieties of *M. esculenta* (P < 0.05). Few reports exist on the antioxidant activity of leaves of *M. esculenta* and *M. glaziovii*. However, to the best of our knowledge, leaves of *M. glaziovii* are only consumed in the west of Democratic Republic of Congo. This species is particularly used elsewhere as biomass for bioethanol and bio-gas production [25]. The antioxidant activity of the other vegetables is also considerable and comparable to results reported in similar studies on ethnic foods from Kwango. *Entada gigas* (L.) Fawc. & Rendle, *Psophocarpus scandens* (Endl.) Verdc, *Salacia pynaertii* De Wild, and *Tetrorchidium congolense* J. Léonard, four unconventional green leafy vegetables with high nutritive value consumed to Kenge, showed important antioxidant activities [26]. Interestingly, these vegetables have high protein content specially *S. pynaertii* that constitute the richest vegetable in methionine and cysteine [13]. Methionine and cysteine are sulfur amino acids, essential for the detoxification of cyanogen glycosides implicated in the occurrence of konzo [27].

S. angustifolium showed a remarkable antioxidant activity which can be attributed to the presence of verbascoside, which is also responsible for high antioxidant activity of herbal teas. Herbal teas exhibited antioxidant activity according to the order *Lippia multiflora > Ocimum gratissimum > Lantana montevidensis > Ocimum basilicum*. Herbal tea is a commonly consumed beverage prepared from different parts of plant species other than *Camellia sinensis*. Particularly, extracts of *L. multiflora* are known to have an excellent antioxidant activity related to their abundance of phenylpropanoids such as verbascoside [17, 28].

Antioxidant activity of studied yams was considerable and *Dioscorea alata* was the most active. Bukatuka et al. studied *Dioscorea alata, Dioscorea bulbifera, Dioscorea dumetorum, Dioscorea burkilliana* and *Dioscorea praehensilis* from Kenge, known to be relevant in traditional medicine for diabetes mellitus treatment. These authors reported that they displayed highest radical-scavenging activities and a good antihyperglycemic activity related to their appreciable

amount of total phenolic contents [29]. Interestingly, cassava flours exhibited higher antioxidant activity than cassava roots. This could be explained by possible chemical modifications during processing before cassava flour is traded. Nevertheless, it is probable that the antioxidant capacity of cassava flours is not sufficient to counteract oxidative damage induced by cyanogenic glycosides. In this context, the mixture of cassava flours with maize, proposed to reduce the ingestion of cyanogenic glycosides from cassava and to improve amino acid intake, could be interesting to promote [8].

Altogether, the antioxidant activities measured with cell-based assays were in good accordance to radical-scavenging capacities. Mushrooms exhibited a considerable cell-based antioxidant activity comparable to certain vegetables. To the best of our knowledge this is the first report regarding the potential inhibitory effect on intracellular ROS production by inflammatory cells of the studied mushrooms and some vegetables such as *D. praehensilis*, *M. glaziovii*, and *S. angustifolium*. Globally, herbal teas showed the highest antioxidant and radical scavenging capacities, followed by vegetables, yams, mushrooms, spices and cassava flours. TLC and HPLC fingerprints of extracts of investigated foods revealed that they contain a diversity of phenolic compounds (Flavonoids, phenolic acids…) [8].

Flavonoids are a group of phenolic compounds or secondary metabolites that are widely distributed in higher plants and are part of our daily diet. It has been reported that flavonoids exhibit a wide variety of biological effects, including anti-inflammatory, anti oxidant, antiviral, antibacterial, anticarcinogenic, antituberculosis, vasodilatory, and antiallergic activities [12]. They are also cytoprotective in various organs and promote intracellular signals that enhance cell survival, among other benefits. However, interest in flavonoids stems mainly from their antioxidant activities, resulting from the catechol group in the B ring, which confers free radical-scavenging activity. Additionally, they act as electron donors or chelators of metal ions (e.g., iron, copper), inhibiting the oxidation of low-density lipoproteins (LDLs). Flavonoids have thus become key compounds. When ingested in the diet, they may prevent and combat neurodegenerative diseases such as Alzheimer disease (AD). Studies have reported that the oral administration of some flavonoids (apigenin, rutin, myricetin…) to mice prevents the development of Alzheimer disease [12]. Rivadeneyra-Domínguez et al. reported that *G. biloba* extract exert a protective effect against behavioral and neuronal damage associated with consumption of cassava juice in the rat and these effects are possibly related with flavonoids [30].

Traditional foods studied contained glycosylated flavonoids mainly the derivatives of quercetin. Quercetin is the major flavonoid in our daily diet and its estimated daily intake is between 5 and 40 mg. After absorption, quercetin is mainly metabolized in the intestine and liver. The plasma concentration of quercetin is normally in the nanomolar range, but it can reach the micromolar range after consumption of quercetin- rich foods [12]. Quercetin is the most extensively studied flavonoid that has been shown to exhibit antioxidant, antiviral, antibacte- rial, anti-inflammatory, and anticarcinogenic properties. Quercetin modulate several cellular signaling pathways involved in regulating the antioxidant response, cell survival, apoptosis, and inflammation [12, 31, 32]. Others compounds such as some biflavonoids founded in the seeds as *Garcinia kola* largely consumed to Kahemba, may have anticancer, antimicrobial, anti-inflammatory, antiviral,

and antimalarial activities [33]. Biflavonoids are compounds with therapeutic potential against AD and other neurodegenerative diseases [12].

Verbascoside is well known for its numerous biological activities including anti-oxidative, anti-apoptosis and anti-inflammatory effects. The *in vivo* effects of verbascoside could also be assigned to its metabolites such as caffeic and ferulic acids [34]. Verbascoside is able to reverse some of the cognitive impairment and to prevent the neuronal apoptosis due to oxidative stress. For this, previous results support the use of traditional medicinal herbs containing acteoside for neuroprotection [35].

Typical preparation methods applied to these food items before consumption include a strong heating process to prepare different sauces with spices and palm oil, which are consumed together with a maize or cassava preparation. Our results are limited to non-cooked traditional foods. The literature reports controversial effects of heat treatment on the antioxidant capacity depending on the analyzed plant and the applied heat treatment. Tsumbu et al. reported that the moderate heat treatment of the green vegetables did not modify their antioxidant and anti-inflammatory capacities [36]. Cooking could lead to the loss of phenolic compounds due to their good solubility in water [37]. However, Ola *et al.* (2009) demonstrated that almost all the phenolic constituents of *M. esculenta* leaves are stable even after heating processes such as boiling [38]. Abdullah *et al.* (2012), reported that selected culinary-medicinal mushrooms extracted by boiling in water for 30 min, showed a good antioxidant activity related to synergistic effects of entire water-soluble fractions [39]. Nevertheless, this aspect should be examined in further studies.

4.3. Anti-inflammatory activity

Motor and cognitive performance continues to be significantly impaired in konzo are associated in part with exposure to poorly processed cassava as measured by urinary thiocyanate [11]. Presence of very high concentrations of thiocyanate (SCN^-), the major metabolite of cyanide, in the bodily fluids of konzo subjects is a consequence of dietary exposure to cyanide. Besides chloride, myeloperoxidase (MPO) also uses the thiocyanate as a major physiological substrate. The SCN^- concentration is a powerful driver of the extent of thiol proteins oxidation in induced by MPO [40, 41] and leading to carbamoylation of proteins [42]. Cassava consumption is associated with increased protein carbamyolation and neurological complications. Prevention of carbamoylation may protect against the neuropathic effects of cyanate [43, 44]. MPO generates a battery of highly diffusible reactive oxidants such as hypochlorite, tyrosyl radicals and aldehydes, which instigate oxidative damage in the host tissues at the inflammatory sites exacerbating tissue damage. In some acute and chronic pathologies, the uncontrolled stimulation of neutrophils could contribute to amplify or maintain the inflammatory response with the release of MPO, a pro-oxidant enzyme involved in secondary cell damage and considered as a marker of inflammation [19]. Indeed, recent investigations have increasingly revealed the cause-effect relationship between MPO and the development of diverse inflammatory diseases supporting MPO and its metabolites as a promising biomarkers not only for infectious diseases but also for a wide array of non-infectious and neurodegenerative disorders [45]. Malle et al. (2007), suggested that the inhibitots of MPO activity are promising therapeutic agents [46].

All plant extracts tested and isolated phenolic acids exhibited a dose-dependent inhibitory effect on MPO activity performed with SIEFED (**S**pecific **I**mmunological **E**xtraction **F**ollowed by **E**nzymatic **D**etection) technic. The SIEFED method used to measure MPO activity allowed the detection of compounds that have a direct interaction with the MPO. For the Hibiscus, dichloromethane extracts showed a stronger inhibition of MPO in comparison to methanolic extracts in the following order: *H. cannabinus* > *H. acetosella* > *H. sabdariffa*. The dichloromethane allowing a better extraction of lipophilic molecules may allow a better interaction of these molecules with the hydrophobic pocket at the entrance of the active site of MPO [16]. Tsumbu et al. evaluated the anti-oxidant, anti-radical, anti-inflammatory, and modulating properties of in "inflammation like" conditions of green vegetables from Bas Congo in DRC [36]. These authors showed that *Abelmoschus esculentus*, *Hibiscus acetosella*, *Manihot esculenta* and *Pteridium aquilinum* were active to inhibit MPO activity and the best effects were observed for *Pteridium* which contains the highest amount of total polyphenols and tannins, and *Manihot*, which has a high content of flavonoids [36]. Caffeoyl-hydroxycitric acid and neochlorogenic acid isolated from Hibiscus species, are less efficient MPO inhibitors in comparison to gallic acid compared to gallic acid, caffeoyl-hydroxycitric acid and neochlorogenic acid are larger molecules that cannot enter easily into the active site of MPO and thus inhibit the enzyme [16]. Gallic acid and caffeic acid were less active than quercetin. Quercetin shown the best activity than his glycosylated flavonoids [8].

Although many phytochemicals present in plant foods are poorly absorbed and undergo rapid excretion, they exert anti-inflammatory, antioxidant, and anticarcinogenic effects at realistic doses. Consumption of phytochemicals may also mediate neurohormetic response through the modulation of adaptive stress-resistance genes, which are responsible for encoding protein chaperones that favor resistance to cellular stress and modulate immune function. Thus, regular consumption of phytochemicals from childhood to adulthood may reduce the risk of age related neurological disorders [32].

Polyphenols are promising neuroprotective agents for the treatment of neurodegenerative diseases and act by different mechanism including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning and cognitive function. Evidence for neuroprotection has been provided by *in vitro* studies showing that various polyphenols protect neuronal cells from damage due to oxidative stress, and by *in vivo* animal studies that have shown their ability to protect neurons against oxidative insults [12].

Traditional foods are good source of essential amino acids and minerals especially for children who are exposed to many diseases. The high nutritive value of these traditional vegetables associated with their important antioxidant activities could contribute to a diversification of the diet in konzo's population, and could then provide benefits leading to a protection against oxidative damage under different conditions including konzo.

5. Conclusion and perspectives

The diet of the population of Kahemba is largely dependent on cassava. Biodiversity of the flora of Kahemba constitutes an untapped reserve of traditional food resources that have

considerable potential for antioxidants. However, *in vitro* findings, such as the antioxidant activities we have measured, are of uncertain relevance to the *in vivo* situation in healthy humans. Further studies are needed to evaluate the *in vivo* activity of these traditional foods and particularly in their cooked forms. This could lead to the valorization of traditional foods as functional foods or nutraceuticals with high antioxidant, anti-inflammatory capacities and high quality protein. This may provide benefits to protect the population of Kahemba against oxidative damage under different conditions, including konzo.

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Conflict of interest

The authors declare that they have no conflict of interest.

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