World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Mineral elements analysis and total flavonoids content in the fresh leaves from two varieties of *Hibiscus sabdariffa* L. consumed as vegetable in Lubumbashi (DR Congo)

Cedrick Mutombo Shakalenga [1,*], Henry Manya Mboni [1], Salvator Nsenga Nkulu [2], Gaël Nzuzi Mavungu [3], Cynthia Kibwe Mwenya [1], James Maloba Mwinensenge [4], Valentin Bashige Chiribagula [1], Salvius Bakari Amuri [1], Joh Kahumba Byanga [1] and Victor Ndibualonji Badibanga Bualufu [5]

[1] *Laboratory of Pharmacognosy, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Lubumbashi, B.P. 1825 Lubumbashi, DR Congo*
[2] *Laboratoire d'écologie et restauration écologique du paysage, Faculté des sciences agronomiques, Université de Lubumbashi, B.P. 1825 Lubumbashi, RD Congo*
[3] *Department of Pre-Clinics, Faculty of Veterinary Medicine, University of Lubumbashi, B.P. 1825, Lubumbashi, DR Congo*
[4] *Laboratory of Chemistry, Department of Chemistry-Physics, Teachers' Training College of Lubumbashi, B.P. 1825, Lubumbashi, DR Congo*
[5] *Department of Biochemistry, Faculty of Veterinary Medicine, University of Lubumbashi, B.P. 1825 Lubumbashi, RD Congo.*

World Journal of Advanced Research and Reviews, 2021, 09(01), 147–155

Publication history: Received on 31 December 2020; revised on 06 January 2021; accepted on 07 January 2021

Article DOI: https://doi.org/10.30574/wjarr.2021.9.1.0512

Abstract

Fresh leaves of sorrel (*Hibiscus sabdariffa* L.) are among the most consumed vegetables in the Lubumbashi in the Democratic Republic of Congo (DR Congo). According to the literature, this vegetable shows the chemical variability depending on the culture area. Two varieties of this species, cultivated and consumed in Lubumbashi have not yet been studied for their micronutrient content, of which fruits and vegetables are the main sources. This study investigated the mineral and total flavonoid content of fresh leaves from red and green varieties of *H. sabdariffa* L., consumed as a vegetable in the aforementioned city. Fresh leaves of red and green variety of *H. sabdariffa* were purchased in 10 main markets of the Lubumbashi city. Gravimetric and spectrophotometric methods were used for analyzes of water, mineral and total flavonoids content. Three of the major mineral elements (Na, Ca, Mg) and several trace elements (Cu, Zn, Fe, Mn, Se, Co, Cr) were found in both varieties in elevated quantity in leaves of red variety. These latter were marked by a high content of iron (7 mg/100 g of fresh material) and Manganese (600 µg/100 g of fresh material). Total flavonoids quantification revealed that the leaves of red variety have a high value (28.2 ± 0.3 mg Quercetin Equivalent per g of extract) in total flavonoids compared to the leaves of green variety. Fresh leaves of the red variety of *H. Sabdariffa* consumed in Lubumbashi could be a source of iron for adults and manganese for children.

Keywords: *Hibiscus sabdariffa* L.; Micronutrient; Mineral elements; Total flavonoids; DR Congo; Lubumbashi markets

1. Introduction

Vegetables and fruits are generally known to be the main sources of micro-nutrients such as minerals [1], vitamins [2] and antioxidants [3]. By this composition, the consumption of vegetables is recommended to ensure a balanced diet for children and adults as well as for the elderly [4–6]. Indeed, the mineral elements of vegetables are essential for growth in children and for strengthening bone mass as well as a good progress of physiological metabolism in adults and the elderly [7]. The antioxidants of vegetables are involved in the reduction of the occurrence and complications of diseases

* Corresponding author: Cedrick Mutombo Shakalenga
Laboratory of Pharmacognosy, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Lubumbashi, B.P. 1825 Lubumbashi, DR Congo.

whose pathophysiology is linked to free radicals [8,9]. These are, for example, cardiovascular, cancer, neurodegenerative pathologies and many others [2,10–13]. Given the diversity of micronutrients, it is essential to identify and group vegetables or fruits according to the types of micronutrients they contain. This article is particularly interested in mineral elements whose lack hinders the growth of children, especially in developing countries [7]; as well as total flavonoids which are very interesting for their antioxidant power playing an important role against diseases due to oxidative stress [14].

In Lubumbashi, the list of vegetables consumed includes different plant species among which we can cite in particular the leaves of amaranths (*Amaranthus hibridus* L.), sweet potato (*Ipomea batatas* (L.) Lam.), cabbages (*Brassica rapa* subsp*. chinensis* (L.) P.Hanelt.; *Brassica oleracea var. capitata* L.), Squash (*Cucurbita maxima* Duchesne.), chard perry (Spinacia olerace L.), and sorrel or Ngaï-ngaï (Swahili) (*Hibiscus sabdariffa* L.) [15–17]. Indeed, the fresh leaves of *H. sabdariffa* are among the vegetables most consumed in Lubumbashi and cited in the bibliography as being of great nutritional value, but whose chemical composition shows great variability depending on the medium of their culture [18]. Actually, *H. sabdariffa* is cultivated in all tropical and semi-tropical regions. In tropical Africa, it is known in particular in the savannah zones of West and Central Africa [19]. The species exists in two varieties, one with green leaves and the other with red leaves [20]. These varieties are also grown in Lubumbashi in the vegetable garden and farms, for local consumption of the leaves.

Some studies have already been carried out along these lines, on some of the vegetables most consumed in Lubumbashi [15]. Another study carried out in Lubumbashi in 2015 showed the anti-sickle cell properties of the leaves and calyxes of a wild species of *H. sabdariffa*, also quantifying the content of water and mineral matter, and highlighting the groups of secondary metabolites present [21]. No study has yet been carried out on the nutritional value of *H. sabdariffa* cultivated and consumed in the Lubumbashi city and even in the region of Haut-Katanga. It is in view of the interesting results of the previous study [21], that the fresh leaves were chosen to be the subject of this analysis. The objective of this study is to carry out qualitative and quantitative analyzes of the mineral elements and the total flavonoids contained in this vegetable, cultivated and consumed in Lubumbashi [16–17]. The interest is to contribute to the knowledge of the vegetables consumed in Lubumbashi but also to provide the population of Lubumbashi with scientific data on the elements which can be brought by the aforementioned vegetable.

2. Methods

2.1. Study area

This study was carried out in the Lubumbashi city, capital of the Haut-Katanga province and located in the southeast of the DR Congo (Figure 1). This city sprawls on a surface of 747 km2, between 11°27’ and 11°47’ South latitude, and between 27°20’ and 27°40’ East longitude, at an average altitude of 1230 m [22]. With an average temperature of 20 °C, Lubumbashi has a tropical climate with two seasons [23]. The dry season lasts from April to October while the rainy season extends from November to March, with an average rainfall of 1150 mm of water [24]. Vegetables consumed in this city are produced mainly by local gardeners or farmers who transport them to different markets [16].

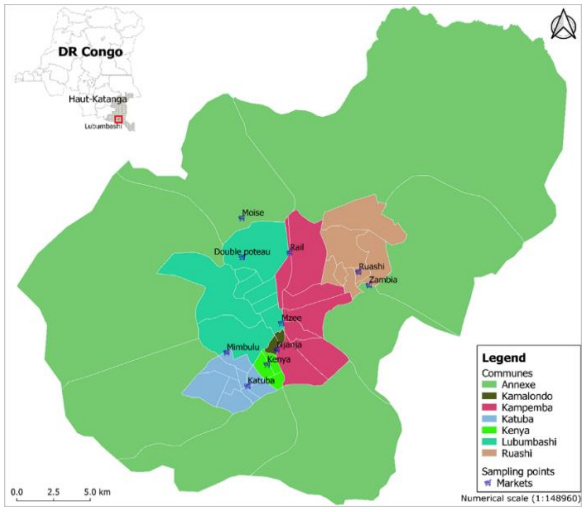

Figure 1 *Study area: Lubumbashi city and geolocation of sample procurement markets. This map has been created by the authors with the QGis 3.12 software.*

Table 1. Geographic coordinates of markets where samples were purchased.

Market name	Administrative address		GPS Location		
	Commune	**Neighborhood**	**Latitude**	**Longitude**	**Altitude**
Double poteau	Lubumbashi	Kalubwe	11°37'42.8"S	27°27'54.2"E	1258 m
Katuba	Katuba	Foyer	11°42'43.2"S	27°28'06.6"E	1205 m
Kenya	Kenya	Luvwa	11°41'53.8"S	27°28'50.4"E	1215 m
Mimbulu	Katuba	Kananga	11°41'25.2"S	27°27'19.5"E	1245 m
Moïse	Annexe	Kasapa	11°36'11.6"S	27°27'53.8"E	1261 m
Mzée	Kampemba	Kinkalabwamba	11°40'17.4"S	27°29'23.0"E	1248 m
Njanja	Kampemba	Kinkalabwamba	11°41'19.5"S	27°29'12.7"E	1238 m
Rail	Kampemba	Cadastre	11°37'32.5"S	27°29'41.4"E	1291 m
Ruashi	Ruashi	Mobutu	11°38'17.1"S	27°32'16.6"E	1318 m
Zambia	Ruashi	Quartier 6	11°38'48.1"S	27°32'40.2"E	1303 m

2.2. Sampling

The fresh leaves of the red and green varieties of *H. sabdariffa* were purchased in June 2016 from ten main markets in the Lubumbashi city (Table 1). Purchases were made for 2 days given the dispersion of the markets. Leaves were stripped of the inedible parts and washed twice with distilled water. Then, these leaves were mixed according to their varieties, to constitute two parent samples (of green and red varieties). Each parent sample was divided into 2 subsamples of each variety. Then, a subsample of each variety was brought to the pharmacognosy laboratory of the Faculty of Pharmaceutical Sciences at the University of Lubumbashi, for the determination of total flavonoids. The remaining sub-samples were brought to the mineral analysis laboratory of the Congolese Office of Control, for the analysis of mineral elements.

Figure 2 *Leaf samples of red and green varieties of H. sabdariffa, purchased from 10 main markets in Lubumbashi*.

2.3. Analysis of mineral elements

The mineral elements were identified and quantified by inductively coupled plasma atomic emission spectrophotometry [25]. Briefly, five grams of each sample, placed in a crucible (High shape porcelain crucible 80ml diam.55mm h.63mm) of known weight, were dried in an oven at 105 °C (Memmert™, Temperature Uniformity ≤±2 °K, Vacuum Range 0.01 mbar, Temperature Metric 5 °C to 200 °C) until constant weight [25]. The sample was then weighed (AND, GR-200, max 210g, min 10mg, e = 1mg, d = 0.1mg) to calculate the water content, then calcined in a muffle oven at 600 °C during 4 h (Furnace mLs1200, Thermo Scientific, Madrid, Spain).

The residues consisting of mineral matter (MM) were weighed, then treated with 15 ml of concentrated nitric acid (70% HNO3, Sigma– Aldrich, Saint Louis, USA), for the mineralization [26]. The mixture was then heated to a gentle boil, until a clear solution is obtained. The latter solution was placed under the peristaltic pump of the atomic emission spectrophotometer (ESO ICP, Perkin Elmer 4300 DV; resolution 0.006 – 200 nm; sensitivity 0.03 – 1 ppm), which draws it. The concentrations in mg/100 g of the analyzed mineral elements was finally printed via the computer coupled to the ESO ICP.

Each sample have been analyzed 3 times and the average value were calculated for each mineral element. The concentration in mg/100 g of mineral elements has been deducted in mg or µg/100 g of fresh material (FM), in order to properly compare the concentration in the studied samples and the daily requirement of each mineral element. This conversion of values was carried out using the equation: CFM = A x B/100. With CFM: Concentration of mineral element in the fresh material; A: concentration of the element in total mineral mater (MM) and B: percentage of total mineral mater in the fresh material (FM). The water and MM contents made it possible to calculate that of organic matter (OM) using the formula: OM = 100 - (% of MM + % of Water).

2.4. Determination of total flavonoids

The extraction of total flavonoids was carried out by maceration with acetone, a solvent which is said to have great extracting power on this group of compounds [27]. The fresh leaves of each variety were pulped and then macerated for 24 hours in 98% acetone (179973, Sigma–Aldrich, Laboratory Reagent, ≥ 99.5%), in a drug/solvent ratio of 1/10 (10 g of drug in 100 ml of solvent) [27]. The extracts were filtered, freed of solvent and kept in the fridge before and during the rest of the manipulations. The flavonoids were first highlighted by the solution staining reaction [28]. In fact, 1 ml of each extract, taken before the solvent evaporation, was mixed with 1 ml of concentrated hydrochloric acid (40% HCl, Sigma–Aldrich, Saint-Quentin Fallavier, France), a few magnesium shavings, and a few drops of iso-amyl alcohol (W205710, natural, ≥ 98%, FG). The formation of the orange-red color in the supernatant testified the presence of flavonoids [21].

Total flavonoid content was measured using the aluminum chloride reagent ($AlCl_3$; 237051, ReagentPlus®, 99%) and quercetin (Q4951, Quercetin, Reference Standard, ≥ 95%, HPLC), used as the reference flavonoid. Aluminum chloride ($AlCl_3$) forms a yellow flavonoid-aluminum complex, with a maximum absorption at 430 nm [29]. Briefly, 1 ml of the sample prepared at 1 mg/ml in methanol [30] is mixed with 1 ml of a methanolic solution of $AlCl_3$ (2%). After 15 min of incubation in the dark, the absorbance is measured at 430 nm by a UV-visible spectrophotometer (UV-2600i, UV-2700i: UV-VIS). Quercetin was prepared in methanol, at respective concentrations of 0.5; 1; 1.5; 2 and 2.5 mg/ml, then treated in the same way as the extracts. The results were calculated from a calibration curve established with the concentrations and absorbance of quercetin and expressed in mg of Quercetin Equivalent (QE) per g of the extract according to the equation included in the figure 2 [25].

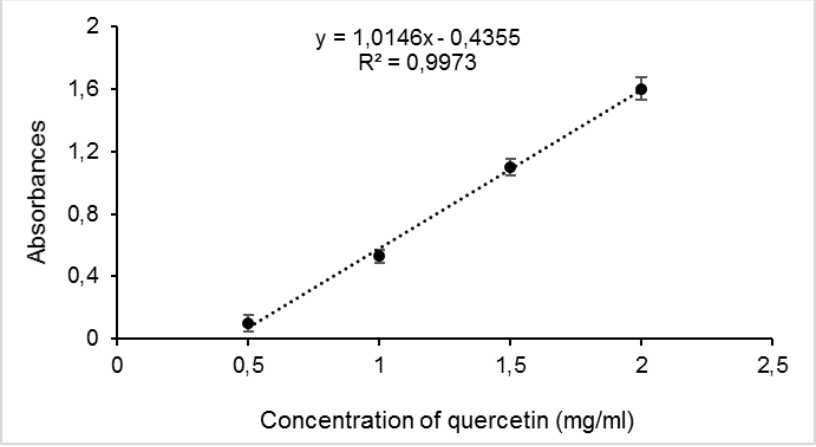

Figure 3 Calibration curve obtained with absorbance of a reference flavonoid (quercetin).

2.5. Statistical data processing

Univariate descriptive statistical analyzes were used to calculate the means and standard deviations of the values of the analyzed elements and present them in the tables. These treatments were carried out with Excel Software (Microsoft Corporation, Washington, USA). The comparison of the means (of the green and red leaves) was carried out by Student's test, using the RStudio.4.0 software. The conditions were checked before application and residual normality was

checked by the Shapiro-Wilks test and homoscedasticity by the Barlett test. The confidency level and error margin were fixed to be 0.95 and 0.05 respectively.

3. Results

The desiccation has shown that the water content of the leaves from the red and green varieties of *H. sabdariffa* is 85 ± 0.5% and 82.5 ± 1.2% respectively (Figure 4). These humidity rates were not significantly different in the analyzed varieties (p = 0.11189). The mineral matter (MM) contents are 1.2 ± 0.1% in the leaves of the red variety and 0.7 ± 0.1% in the leaves of the green variety. These results show a high MM content in the leaves of the red variety since the difference is statistically significant (p = 0.0255). The value of water content and mineral mater made it possible to calculate the OM content which amounts to 14.1 ± 0.2% in the leaves of the red variety and 14.1 ± 0.1% in the leaves of the green variety. As these values are not significantly different (p = 0.8593), the organic matter (OM) content is similar in these two varieties (Figure 4).

Mineral analysis showed the presence of ten elements including 3 macro elements and 7 trace elements in the leaves of the two varieties of *H. sabdariffa*; with values generally high in the leaves of the red variety. Calcium (7.6 and 3.4 mg), iron (7.1 and 2.3 mg), manganese (600 and 200 µg) and zinc (29 µg and 100 µg) were most abundant in 100 g of fresh material of leaves from the red and green respectively (Table 2).

Total flavonoid compounds have been qualitatively demonstrated in both varieties. Their quantification showed contents of 28.2 ± 0.3 mg of QE/g of extract from the red variety and 26.4 ± 0.3 mg of QE/g of extract from green variety. The comparison of these values showed that the red leaves contain a higher quantity, statistically different from that found in the green leaves (p = 0.0085).

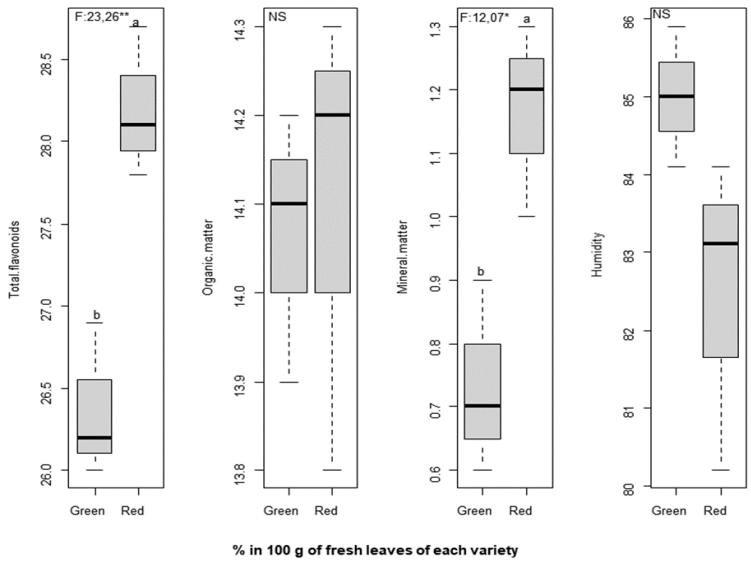

Figure 4 *Statistical comparisons of the water contents, organic matter, mineral matter and total flavonoids. F: Student test value (t_0); a: high value; b: lower value; NS: no significant difference; *: significant difference ($p < 0.05$); **: very significant difference ($p < 0.005$).*

Table 2 Mineral elements found in fresh leaves of *H. sabdariffa* consumed in Lubumbashi

Found mineral elements		**Green variety MM=0.6 ± 0.04 % of fresh material (FM)**		**Red variety MM=1.3 ± 0.02 % of fresh material (FM)**	
		in 100g of MM	**in 100g of FM [a]**	**in 100g of MM**	**in 100g of FM [a]**
Macro elements	Calcium	560.7 ± 2.3 mg	3.4 mg	583.6 ± 1.7 mg	7.6 mg
	Sodium	69.1 ± 0.2 mg	0.4 mg	54.2 ± 1.0 mg	0.7 mg
	Magnesium	206.9 ± 0.1mg	1.2 mg	240.4 ± 2.2 mg	3.1 mg
Trace elements	Chrome	0.6 ± 0.02 mg	4 µg	0.7 ± 0.01 mg	9.0 µg
	Copper	3.2 ± 0.3 mg	19 µg	3.6 ± 0.02 mg	47.0 µg
	Iron	375.8 ± 0.4 mg	2.3 mg	548.7 ± 1.4 mg	7.1 mg
	Manganese	38.7 ± 1.3 mg	200 µg	43.6 ± 2.0 mg	600.0 µg
	Cobalt	4 ± 0.7 µg	0.02 µg	6 ± 0.3 µg	0.1 µg
	Selenium	0.2 ± 0.01 mg	1 µg	0.3 ± 0.01 mg	4 µg
	Zinc	4.8 ± 1.1 mg	29 µg	5.7 ± 0.4 mg	100 µg

MM: Mineral matter; FM: Fresh matter

a: these values were calculated considering the average without standard deviations

4. Discussion

This study is the first to show the mineral elements and the level of total flavonoids present in the leaves of *H. sabdariffa* consumed in the Lubumbashi city. Indeed, the leaves analyzed in this study contain water and OM contents located within the limits of fresh leafy vegetables [31]; the water contents are close to that found by Mulungulungu and Badibanga in 2015 in the leaves of *H. sabdariffa*, in Lubumbashi [21]. The MM contents observed in this study (Figure 4) is lower than those found by the aforementioned authors (7.3%); however, it is equivalent to that observed by Morton in Guatemala (1%) [32]. The difference in the quantity of mineral matter observed in this study and that reported by Mulungulungu and Badibanga can be justified by the fact that the content of mineral elements presented by the aforementioned authors was calculated on the dry matter, whereas it was calculated on fresh matter in this study.

The difference in MM content observed between the two studied varieties is probably due to the difference in the assimilation capacity of mineral elements [33,34]. The multitude of mineral elements found in the leaves of *H. sabdariffa* is in agreement with Cisse *et al*. [18], according to which this species is a source of mineral elements. The comparison of the contents of mineral elements found in 100 g of each sample and the daily needs for a child and an adult showed that the values all mineral elements found in the leaves of the green variety are below the daily needs of both a child and an adult [5,35–43]. Otherwise, the leaves of the red variety have levels corresponding to the daily iron requirements for an adult (6 mg) [35] and manganese for a child (500 µg) [36]. Thus, the consumption of at least 100g of leaves of the red variety would be favorable to any person, being the quantities of varied mineral elements which they bring. Indeed, a study carried out in Ghana showed that women of childbearing age fed a meal made from *H. sabdariffa*, at the frequency of 3 meals per week, for 12 weeks saw an improvement in their iron status compared to the control group [44].

Iron is required for oxygen transport, electron transfer, oxidase activities and energy metabolism [35]. The richness of the leaves the red variety in iron is related to the hematopoietic properties attributed to the leaf of this variety [44]. In addition, leaves of red variety can be recommended for iron-deficient adults, such as pregnant or lactating women [45,46]. Let us add that the difficulty of intestinal absorption of phytic iron (Fe^{3+}), would be reduced in this vegetable by the presence of ascorbic acid [18] which reduces it to ferrous iron (Fe^{2+}), absorbable at the intestinal level [47]. Manganese is an essential dietary element for mammals. It is a component of metalloenzymes such as superoxide dismutase, arginase and pyruvate carboxylase, and is involved in amino acid, lipid and carbohydrate metabolism [36]. Manganese deficient children can receive red leaves as a supplement to the basic diet.

The presence of flavonoids in the leaves of *H. sabdariffa* has also been reported by Mulungulungu and Badibanga, however the concentration of flavonoids reported by these authors (22%) [21] is lower than that found in this study

regardless of the variety considered. This difference would probably be due to that of the quantification methods used, although the aforementioned authors have not explained their means of quantifying total flavonoids. In addition, the presence of flavonoids in the analyzed leaves, would be explained by their role as sunscreen, the role of which are those organs exposed to the sun [48]. The difference observed between the quantities of total flavonoids in the analyzed varieties could be due to their genotypic difference [49]. According to Escobar-Cévoli *et al*. [50], the daily need for total flavonoids for human is estimated between 50 and 150 mg depending on the country. This implies that the total flavonoids content observed in the studied vegetable (26.4 ± 0.3mg in the green variety and 28.2 ± 0.3 mg in the red variety), are less than the person's daily requirement. However, the presence of these antioxidant in this vegetable is a benefit, as they would help limit or neutralize the effects of free radicals in people consuming these fresh leaves [6,11]. On the other hand, the high amount of total flavonoids in the red leaves can be considered as an added value to its richness in iron and manganese.

5. Conclusion

This study reveals the mineral and total flavonoid content in the leaves of the red and green variety of *H. sabdariffa* consumed as vegetable in the Lubumbashi city, and shows that the consumption of 100 g of the leaves of red hibiscus can cover the daily requirements of iron and manganese for an adult and a child respectively. She suggests that analyzes continue on these two vegetables to complete the information related to their nutritional value.

Compliance with ethical standards

Acknowledgments

The authors sincerely thank the Congolese control office for the mineral analyzes

Disclosure of conflict of interest

The authors declare no competing interests

References

[1] Mohammed MI, Sharif N. Mineral composition of some leafy vegetables consumed in Kano, Nigeria. Niger J Basic Appl Sci. 2011; 19: 208–12.

[2] FAO, WHO. Human vitamin and mineral requirements. Report of a Joint FAO/WHO Expert Consultation Bangkok, Thailand: Food and Nutrition Division FAO. 2001.

[3] Shetty A, Magadum S, Managanvi K. Vegetables as Sources of Antioxidants Journal of Food & Vegetables as Sources of Antioxidants. J Food Nutr Disord. 2013; 2: 1000104.

[4] Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants : a review. African J Food Sci. 2010; 4: 200–22.

[5] EFSA. Scientific opinion on dietary reference values for calcium. EFSA J. 2015; 13: 4101.

[6] Alkhalidy H, Wang Y, Liu D. Dietary flavonoids in the prevention of T2D : an overview. Nutrients. 2018; 10: 1–33.

[7] Bailey LR, Keith PWJ, Black RE. The epidemiology of global micronutrient deficiencies. J Nutr Metab. 2015; 66: 22–33.

[8] Perrone S, Santacroce A, Longini M, Proietti F, Bazzini F, Buonocore G. The free radical diseases of prematurity : from cellular mechanisms to bedside. Hindawi Publ Corp. 2018; 1–14.

[9] Florence TM. The role of free radicals in disease. Ida Mann Lect. Present. 24th Sci. Congr. R. Aust. Coll. Ophthalmol., Sydney. 1992; 3–7.

[10] Duarte J, PeÂrez-Palencia R, Vargas F, Ocete MA, Pérez-Vizcaino F, Zarzuelo A, et al. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Br J Pharmacol. 2001; 133: 117–24.

[11] Moline J, Bukharovich IF, Wolff MS, Phillips R. Dietary flavonoids and hypertension : is there a link ? Med Hypothesis. 2000; 55: 306–9.

[12] Abotaleb M, Samuel SM, Varghese E, Varghese S, Kubatka P, Liskova A, et al. Flavonoids in cancer and apoptosis. Cancer. 2019; 11: 1–39.

[13] Al-ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D. Flavonoids and Their Anti-Diabetic E ff ects : Cellular Mechanisms and E ff ects to Improve Blood Sugar Levels. Biomolecules. 2019; 9: 1–35.

[14] Adwas AA, Sedik A, Elsayed I, Azab AE, Quwaydir FA. Oxidative stress and antioxidant mechanisms in human body. J Appl Biotechnol Bioeng. 2019; 6: 43–7.

[15] Ndibualonji BB V, Pemba MG, Maryabo KG, Kaputo M. Évaluation de la valeur nutritive et recherche des substances bioactives des feuilles de deux variétés de patate douce consommées à Lubumbashi (RDC). J Appl Biosci. 2016; 102: 9771–6.

[16] Mubemba MM, Useni S, Mwamba M, Kateta MG, Mwansa M, Ilunga K, et al. Teneurs en éléments traces métalliques dans les sols de différents jardins potagers de la ville minière de Lubumbashi et risques de contamination des cultures potagères. J Appl Biosci. 2013; 66: 5106–13.

[17] Mutshail MG. Projet d'appui au développement de l'horticulture urbaine et periurbaine (HUP) à Lubumbashi (RDC). SENAHUP/PROJET HUP-FAO, Lubumbashi. 2000.

[18] Cisse M, Dornier M, Sakho M, Ndiaye A, Reynes M, Sock O. Le bissap (*Hibiscus sabdariffa* L.) : composition et principales utilisations. Fruits. 2009; 64: 179–93.

[19] Amusa NA, Adegbite AA, Oladapo MO. Vascular Wilt of Roselle (*Hibiscus sabdariffa* L. var. sabdariffa) in the Humid Forest Region of South-western Nigeria. Plant Pathol J. 2005; 4: 122–5.

[20] Cisse M, Dornier M, Sakho M, Diop CMAR, Reynes M, Sock O. La production du bissap (*Hibiscus sabdariffa* L.) au Sénégal. Fruits. 2009; 64: 111–24.

[21] Mulungulungu ND, Badibanga ML. Etude des propriétés antidrépanocytaires et de la thermo dégradation des colorants extraits de l'Hibiscus Sabdariffa, plante légumière à vertu thérapeutique. Int J Innov Appl Stud. 2015; 10: 384–92.

[22] Kasongo MP, Mukoko KG, Kipata ML, Lunda IJ-M. Elaboration de la carte géotechnique de la ville de Lubumbashi guide technique de sélection des sites d'implantation d'ouvrages du génie civil. Eur Sci J. 2018; 14: 1857–7881.

[23] Mutondo TG, Kamutanda KD, Numbi MA. Evaluation du bilan hydrique dans les milieux anthropisés de la forêt claire (région de Lubumbashi, Province du Haut-Katanga, R.D. Congo). Méthodologie adoptée pour l'estimation de l'évapotranspiration potentielle. Geo Eco Trop. 2018; 42: 159–72.

[24] Van de Vyver H, Demarée GR. Construction of Intensity–Duration–frequency (IDF) curves for precipitation at Lubumbashi, Congo, under the hypothesis of inadequate data. Hydrol Sci J. 2010; 55: 555–64.

[25] Bashige C V, Bakari AS, Okusa NP, Kahumba BJ, Lumbu SL. Potential nutrition of leaves and fruits of *Dialium angolense* Welw. ex Oliv. (Fabaceae) an antimalarial plant from the Eastern of DR Congo. Int J Adv Res. 2020; 8: 336–45.

[26] Würfels M. Voltammetric determination of metal traces in marine samples after nitric acid decomposition. Mar Chem. 1989; 28: 259–64.

[27] Mahoudi S, Khali M, Mahmoudi N. Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (*Cynara scolymus* L.). Nat Technol. 2013; 9: 35–40.

[28] Chandler B, Harper K. Identification of saccharides in anthocyanins and other flavonoids. Aust J Chem. 1961; 14: 586–95.

[29] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food Chem. 2006; 97: 654–60.

[30] Nickavar B, Esbati N. Evaluation of the antioxidant capacity and phenolic content of three thymus species. J Acupunct Meridian Stud. 2012; 5: 119–25.

[31] Okouango YSI, Michel E, Moutsamboté JM, Mananga V, François M. Évaluation de la consommation et de la composition nutritionnelle des légumes-feuilles de *Phytolacca dodecandra* l'Herit consommés par les populations originaires des districts d'Owando et de Makoua. J Anim Plant Sci. 2015; 27: 4207–18.

[32] Morton JF. Roselle, *Hibiscus sabdariffa* L. In: Morton JF, editor. Fruits Warm Clim, Miami. 1987; 281–6.

[33] Lopez-Arredondo DL, Leyva-Gonzalez, Marco A, Alatorre-Cobos F, Herrera-Estrella L. Biotechnology of nutrient uptake and assimilation in plants. Int J Dev Biol. 2013; 57: 595–610.

[34] Pathak N, Agrawal S. Atomic absorption spectrophotometer analysis for determination of variation in mineral content in Fenugreek genotypes cultivated at three different locations. Int J Pharm Sci Invent. 2014; 3: 40–5.

[35] EFSA. Scientific opinion on dietary reference values for iron. EFSA J. 2015; 13: 4254.

[36] EFSA. Scientific opinion on dietary reference values for manganese. EFSA J. 2013; 11: 3419.

[37] EFSA. Scientific opinion on dietary reference values for magnesium. EFSA J. 2015; 13: 4186.

[38] EFSA. Dietary reference values for sodium. EFSA J. 2019; 17: 5778.

[39] EFSA. Scientific opinion on dietary reference values for chromium. EFSA J. 2014; 12: 3845.

[40] EFSA. Scientific opinion on dietary reference values for copper. EFSA J. 2015; 13: 4253.

[41] EFSA. Scientific opinion on dietary reference values for zinc. EFSA J. 2015; 12: 3844.

[42] EFSA. Scientific opinion on dietary reference values for selenium. EFSA J. 2014; 12: 3846.

[43] EFSA. Scientific opinion on the use of cobalt compounds as additives in animal nutrition. EFSA J. 2009; 7: 1383.

[44] Kubuga CK, Hong HG, Song WO. *Hibiscus sabdariffa* meal improves iron status of childbearing age women and prevents stunting in their toddlers in northern Ghana. Nutrients. 2019; 11: 1–12.

[45] Cogswell ME, Parvanta I, Ickes L, Yip R, Brittenham GM. Iron supplementation during pregnancy, anemia, and birth weight: a randomized controlled trial. Am J Clin Nutr. 2003; 78: 773–81.

[46] Kalenga M-K, Nyembo M-K, Nshimba M, J.-M. F. Étude de l'anémie chez les femmes enceintes et les femmes allaitantes de Lubumbashi (République Démocratique du Congo) Impact du paludisme et des helminthiases intestinales. J Gynécologie Obs Biol La Reprod. 2003; 32: 647–53.

[47] Cook JD, Reddy MB. Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr. 2001; 73: 93–8.

[48] Samanta A, Das G, Das KS. Roles of flavonoids in Plants. Int J Pharm Sci Technol. 2011; 6: 1–25.

[49] Josipović A, Sudar R, Sudarić A, Jurković V, Kočar MM, Kulundžić AM. Total phenolic and total flavonoid content variability of soybean genotypes in eastern Croatia. Croat J Food Sci Technol. 2016; 8: 2–7.

[50] Escobar-Cévoli R, Castro-Espin C, Béraud V, Buckland G, Zamoras-Ros R. An overview of global flavonoid intake its food sources. *In* Justino GC [ed]. Flavonoids - From Biosynthesis to Human Health. IntechOpen, London. 2017; pp 371–391.