Research Article

Journal of Chemistry Letters
journal homepage: www.jchemlett.com
ISSN (online) 2717-1892

Levels of Heavy Metals and its Health Risk Estimation in Calyces of Red and White Roselles Purchased from Monday-Market, Borno State

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ARTICLE INFO

Article history:
Received 5th December, 2021
Received in revised form 25th December, 2021
Accepted
Available online

Keywords:
Roselle,
Heavy metals,
Hibiscus,
Risk Assessment,
Cancer Risk.

ABSTRACT

Heavy metals accumulation in vegetables and their jeopardous health effects have been on increase especially after a long-term exposure. This study examines the levels of some heavy metals in calyces of red and white roselle obtained from Monday-Market, Maiduguri, Borno state, Nigeria. Furthermore, the study estimates the health risk associated with consumption of the calyces. The calyx samples were prepared in accordance with standard procedures and subjected to smart spectrophotometer for heavy metal analysis. The variation trend of the heavy metals in the red roselle was Cu > Zn > Cr > Cd while in the white roselle was Zn > Cu > Cr > Cd. However, Hg was not detected in both samples. The Health Quotient (HQ) calculated for the heavy metals showed that Cd and Cu were greater than unity indicating that consumers may be exposed to potential non-carcinogenic health risk due to Cd and Cu intoxication. The Target Cancer Risk (TCR) values (2.26×10^{-4} - 1.34×10^{-1}) recorded in the present study were all above the allowable range of $1 \times 10^{-6} – 1 \times 10^{-4}$ by United Nations Environmental Protection Agency (USEPA) indicating cancer risk through ingestion of these calyces. The risk of cancer among the metals for both samples followed the sequence Cu > Cr > Cd.

1. Introduction

Heavy metals contamination is a major problem of our environment and they are also one of the major contaminating agents of our food supply. The knowledge of metals in food is essential for assessing the dietary intakes of essential metals and human exposure to toxic elements [1]. The non-biodegradable nature, long biological half lives and their ability to proliferate in various body parts make heavy metals dangerous pollutant. Consumption of foods contaminated with heavy metals for a long period can lead to their accumulation in the kidney and liver of human beings resulting in different dysfunctions in many biochemical processes that could result in kidney, bone, cardiovascular and nervous diseases [2]. *Hibiscus sabdariffa L.* is known as roselle or red roselle in English, Karkade in Arabic, Karasu in Kanuri, Yakuwa in Hausa and Amukan in Yoruba languages of Nigeria. It is taken as a common local drink popularly known as zobo in Nigeria. It is cultivated for leaf, fleshy calyx, seed or fiber according to the respective properties of the two major varieties *var. rubber* (red) and *var. intermidius* (green). The thick red and fleshy cup-shaped calyces of the flower are consumed worldwide as a cold beverage and as a hot drink (Sour tea) [3]. *Hibiscus sabdariffa L.* is also used in folk medicine against many complaints that include high blood pressure, liver diseases and fever [4,5]. In Nigeria, a decoction of the seed is given to augment or induce lactation in poor let down and maternal mortality [6]. The red colouring makes it popular ingredient of commercial herbal teas. In some places, its leaves are also used as a vegetable and its stem has a fiber that is sometimes used for domestic purposes. Seed of roselle are source of nutrition, protein and other beneficial elements [7]. The seed contains oil which is good as a lubricant fuel, and used for making soap. The physicochemical characteristics of roselle was studied and it was characterized as a highly acidic fruit with low sugar content. Succinic acid and oxalic acid were quantified as two predominant organic acids in roselle. Roselle was found to contain higher amount of ascorbic acid compared to orange and mango [8]. Figure 1 shows the aerial view of the red and white roselle plants.

The aim of the study was to examine the physicochemical properties and levels of some heavy

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metals (Cu, Zn, Cr, Cd, Hg) in the dried calyces of red and white roselle with a view to estimating the average daily intake of the metals thus assessing the human health risk associated with their consumptions.

(a)

(b)

Figure 1. Aerial view of red (a) and white (b) *Hibiscus sabdariffa* plant

2. Results and Discussion

2.*1 Concentrations of Heavy Metals*

The results of the heavy metals determination conducted on both red and white dried calyces of *Hibiscus sabdariffa L.* showed the presence of Zn, Cu, Cr and Cd as presented in Table in 1. However, Hg was not detected in both samples. Mild variations in the concentrations of the studied heavy metals in the red and white calyces of the plant were observed. According to Click and Roscoe [18], dried calyces of *Hibiscus sabdariffa* L. are a rich source of vitamin A, and also stated in their research work that, the presence of metals like Pb, Mn, Cd, Cu, Cr and Zn in the plant calyces is attributable to the presence of the metals in the soil which in turn got transported from the soils to the plants.

Vijaya *et al.*, [19] concluded assessment of heavy metals in roselle samples of certain locations situated around Karnataka, Tumkur, India. The analysis of the heavy metals in the dried calyces revealed that except the sample of Pb which was wet ashed, the concentration of all other elements namely; Mn, Zn, Cu and Cd were within the scope limits for intake into the system as set by WHO. The presence of these heavy metals is an indicator that the plant can be potential source of precursors in the development of synthetic drugs. Several researchers have reported the efficiency of the plant's constituents and vitality.

The mean concentration of Zn was found to be 0.15 mg/kg and 0.31 mg/kg in the red and white calyces respectively. The mean concentrations are lower than 1.22 mg/kg reported by Dio and Madusolumuo [20] but higher than 0.039 mg/kg reported by Sarkiyayi and Samaila [21]. However, the values fall within the range recommended by FAO/WHO [22]. Zinc is known for its normal growth and development functions in human beings. Its inadequacy in the system can lead to impaired absorption, excessive excretion or inherited defects in its metabolism [21]. Its deficiency or excessiveness may enhance susceptibility to carcinogenesis [23].

In this study, the concentration of copper in the red and white calyces was 0.21 and 0.17 mg/kg respectively. These levels are higher than 0.021 mg/kg reported by Sarkiyayi and Samaila [21] but lower than 7.05 – 18.44 mg/kg reported for vegetables [24] Nevertheless, the observed concentrations are also within the acceptable range set by WHO. Copper has high biological effect when taken at higher concentrations which include kidney and liver damage.

The Cd content stood at 0.014 mg/kg and 0.015 mg/kg in the red and white calyces respectively. These concentrations fall within the allowable limit of 0.20 mg/kg set by WHO. The result is in agreement with the level of heavy metals reported for drinks made from the calyx [1] and level of Cd reported in the leaves (0.001 – 0.101 mg/kg) [26] but below 0.73 mg/kg as reported in a work [27]. Contrarily, Cd was not detected in a study carried out on plant grown near lake Geriyo,Yola in Adamawa state of Nigeria [20].

Consumption of food contaminated with chromium may pose serious health hazards such as renal failure, liver and lung dysfunctions and bone defects. Long-term consumption induces or exacerbates diabetes, hypertension and myocardial dysfunction [28,29].The mean concentration of Cr was recorded as 0.02 mg/kg in both red and white calyces. These are in line with the WHO allowable limit of 0.1 mg/kg. The hexavalent chromium is more harmful with effects such as necrosis, membrane ulcer and dermatitis [23].

Table 1. Concentrations of Some Heavy metals in red and white Roselles

Heavy metals	**Red Roselle (mg/kg)**	**White Roselle (mg/kg)**	**FAO/WHO Limit** [22] **(mg/kg)**
Cr	0.020	0.020	0.10
Zn	0.150	0.310	100.00
Cd	0.014	0.015	0.20
Cu	0.210	0.170	73.00
Hg	ND	ND	0.01

ND = Not detected

2.2 Physicochemical Parameters

Table 2 shows the physicochemical parameters of calyces of red and white roselles. The physicochemical parameters such as temperature, alkalinity, conductivity and pH were also measured and found to be within the range accepted by WHO. However, the found pH values of 9.81 and 9.63 for the red and roselles respectively were higher than the allowable range of 6.5 -8.5.

Table 2. Physicochemical properties of calyces of red and white roselles

Parameters	Unit	Red Roselle	White Roselle	WHO Standard
Conductivity	µS/cm	2.37	3.19	400 – 600
pH	-	9.81	9.63	6.5 - 8.5
Alkalinity	mg/l	56.06	85.18	20 – 200
Temperature	ºC	25.00	25.30	30

2.3 Health Risk Quotient (HQ) and Health Index (HI)

The health risk quotients due to daily consumption of the calyces of red and white roselle are presented in Table 3. The highest and least values were respectively recorded for Cd and Cr in both samples. Similar result was also observed for Cd as reported by Ftsun and Abraha [12]. Generally, when HQ < 1, it implies there are no potential health risk associated with the current level of exposure to a given heavy metal and the population under study is considered to be safe [31]. The HQ values for Cr and Zn in the studied samples were lower than 1. However, the estimated HQ values for Cd and Cu ranged from 1.80625 – 6.38 in both roselle varieties. When HQ exceeds 1, it implies there are potential perilous health effects from exposure. The consumers may be exposed to potential non-carcinogenic health risk due to Cd and Cu intoxication. In both roselle calyces studied, the total HQ or HI was far higher than 1 (red roselle: 8.39941, White roselle: 8.63107) and this might overestimate the potential non-cancer health risk [17]. The results are presented in **Table 3.**

Table 3. Average Daily Intake (ADI), Health Quotient (HQ) of the Heavy metals in calyces of red and white Roselles

Heavy metals	ADI (mg/person/day)		HQ		Rf_D (mg/kg/day) [14,16]
	Red Roselle	White Roselle	Red Roselle	White Roselle	
Cr	0.00850	0.00850	0.00566	0.00566	1.50
Zn	0.06375	0.13175	0.21250	0.43916	0.300
Cd	0.00595	0.00638	5.9500	6.38000	0.001
Cu	0.08925	0.07225	2.23125	1.80625	0.04
Hg	-	-	-	-	-

3.4 Target Cancer Risk (TCR)

Table 4 shows the target cancer risk values for human due to consumption of calyces of red and white roselles. If TCR value exceeds 1×10^{-4} is considered as intolerable, the values lower than 1×10^{-6} is considered as safe [17]. The TCR values (2.26×10^{-4} - 1.34×10^{-1}) recorded in the present study are all above the allowable range of 1×10^{-6} – 1×10^{-4} by USEPA indicating cancer risk through ingestion of these calyces [32]. The risk of cancer among the metals for both samples followed the sequence Cu > Cr > Cd.

Table 4. Target Cancer Risk (TCR) of the Heavy metals from consumption of calyces of red and white Roselles

Heavy metals	CPSo (mg/kg/day) [17]	Target Cancer Risk (TCR)	
		Red Roselle	White Roselle
Cr	0.5	4.25×10^{-3}	4.25×10^{-3}
Zn	NF	-	-
Cd	0.38	2.26×10^{-4}	2.42×10^{-3}
Cu	1.5	1.34×10^{-1}	1.08×10^{-1}
Hg	-	-	-

NF = Not found in literature

3. Experimental

3.1 Study Area

Maiduguri, the Borno state capital is located in northeastern Nigeria. The city is located between latitude 11º50'N and longitude 13º09'E. Monday-market is the largest commercial centre in northeastern Nigeria market in the city [9]. The market lies on coordinate 11.83º N and 13.15º E.

3.2 Experiments

3.2.1 Chemicals

Buffered ammonia reagent, Chromium reagent powder, Copper tablet, Formaldehyde solution, Buffer solution, HCl, HNO_3, PAN indicator, Sodium ascorbate powder, Sodium cyanide, Zinc buffer powder and zinc indicator used in the study were all of analytical grade. All preparations were done using distilled water.

3.2.2 Instrumentation

Smart spectro 2 spectrophotometer LaMotte was used for determination of the concentrations of heavy metals in the calyces of red and white roselle samples.

3.2.3 Sampling

The samples, dry calyces of red and white roselle plants were purchased from Monday market, Maiduguri, Borno State and conveyed in polyethene bag to the laboratory for preparation and analysis.

3.2.4 Sample Preparation

Two grams (2.0 g) of the sample was weighed into a crucible and incinerated at 600 ºC in a muffle furnace for 3 hours. The ashed sample was removed from the furnace and cooled in a desiccator. To the ashed sample, 10 ml of 6 N HCl was added and covered with a watch glass, placed on a water bath and boiled for 10 minutes. The sample was removed and filtered into 100 ml volumetric flask. The filter paper was washed down to the volumetric flask and the volume was made up to 100 ml using distilled water [10,11].

3.3 Estimated Average Daily Intake of Heavy Metals (ADI)

The average intake of heavy metals was calculated in accordance with equation (1) as reported [12].

$$\mathrm{ADI} = \frac{\mathrm{Cmetal} \times \mathrm{Cfactor} \times \mathrm{Dfood\ intake}}{Bw} \qquad \textbf{Eqn. 1}$$

Where ADI is the Average Daily Intake, Cmetal is the average concentration of heavy metals in calyces, Cfactor is conversion factor to convert fresh vegetable weight to dry weight (given as 0.085), Dfood intake is the daily intake of vegetables (suggested by WHO guidelines in human diet is 300 – 350 g per person). The mean of 325 g/person/day was used in calculating the ADI values in this study and Bw is average body weight (65 kg).

3.4 Health Quotient (HQ)

The value of health risk index depends on the daily intake of metals and oral reference dose (Rf_D) in $mg^{-1}kg^{-1}persion^{-1}$. Rf_D is an estimated exposure of metal per day to human body that has no harmful effect during life time. The HQ was calculated using equation (2) as reported [13,14].

$$\mathrm{HQ} = \frac{\mathrm{ADI}}{\mathrm{RfD}} \qquad \textbf{Eqn. 2}$$

Where, Rf_D is the reference oral dose and ADI is the daily intake of metals. Human beings are considered safe if the HQ < 1 [14,15].

3.5 Hazard Index (HI)

An exposure to more than one pollutant results in additive effects. Thus, hazard index (HI) is a vital index that assesses overall likely impacts that can be posed by exposure to more than one contaminant. HI > 1, suggests significant health effects from consuming pollutants contained in a food. The HI is calculated as an arithmetic sum of hazard quotients for each pollutant as represented in equation (3).

$$\mathrm{HI} = \sum_{i=1}^{4} \mathrm{HQ} = (\mathrm{HQ(Cr)} + \mathrm{HQ(Cd)} + \mathrm{HQ(Cu)} + \mathrm{HQ(Zn)} + \mathrm{HQ(Hg)}) \qquad \textbf{Eqn. 3}$$

Where HQ is hazard quotient of the respective heavy metals [16].

3.6 Target cancer Risk (TCR)

The target cancer risk is used to estimate the carcinogenic risk of metals for a lifetime. The values were calculated from equation (4).

$$\mathrm{TCR} = \mathrm{CPSo} \times \mathrm{ADI} \qquad \textbf{Eqn. 4}$$

Where, ADI is the average daily intake of heavy metals, CPSo is the oral carcinogenic potency slope values (mg/kg/day) are 0.5, 0.3 and 1.5 for Cr, Cd and Cu respectively [17, 30].

4. Conclusion

This study showed that the concentrations of Cr, Zn, Cd, Cu and Hg determined in the calyces of red and white roselles were within the FAO/WHO allowable limits. The health risk index was estimated for all the metals. The values for Cd and Cu revealed probable exposure of consumers to potential non-carcinogenic health danger. The assessed target cancer risk for the analyzed metals follow the order Cu > Cr > Cd.

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