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A Study of Oxalate Content of Some Selected Species of *Hibiscus* Cultivated in Kwilu State/Democratic Republic of Congo

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Authors' contributions

This work was carried out in collaboration between all authors. Author KSJ designed the study and wrote the protocol. Authors MSO and KTJ managed the analyses of the study and wrote the first draft of the manuscript. Author MKS managed the literature searches. Author NKM performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work aimed to determine the total content of oxalate on some species of *Hibiscus* collected at Kwilu state/Congo-Dr, and to determine the variation of this anti-nutritional factor according to the plant age and after the removal of first cooking water.
Study Design: Different species of *Hibiscus* are as well consumed in Kwilu state (DRC), where they are often dried in order to be consumed during the dry season. To date, it is well known that high-oxalate diets may increase the risk of kidney stones and others health problems in susceptible people.

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Place and Duration of Study: Different samples of *Hibiscus* were collected in rain season (January to April 2016), in Kwilu state (DRC). Laboratory analyses were done at the Department of Chemistry (Lacoren/Unikin).
Methodology: Ten species of *Hibiscus* were collected, and their oxalate content was determined using volumetric titration's method with a standard solution of potassium permanganate.
Results: The results showed significant oxalate content, ranging from 1056.12 +/- 34.43 mg/100 g to 422.40 +/- 23.71 mg/100 g of fresh weight. The elimination of first cooking water reduces this anti-nutritional factor to more than 70%. The oxalate content variation with the age of three selected *Hibiscus* has been investigated which shows a maximum level of high oxalate content between 55 to 60 days of plant development.
Conclusion: A non-negligible concentrations of oxalate were found from the ten species of *Hibiscus* used in this work, regardless to the plant age. The removal of first cooking water may serve to greatly reduce the oxalate content.

Keywords: Hibiscus; total oxalate; first cooking water; plant age; consumption.

1. INTRODUCTION

Oxalic acid is an organic acid found in many fruits and vegetables in variable concentration. Several species of *Hibiscus* have been reported to contain a non-negligible concentration of organic acid [1]. These vegetables are widely consumed in Kwilu state (DRC), where they are often dried in order to be consumed during the dry season. Kwilu State has a sandy soil rich in organic compounds; *Hibiscus* is one of the rainfed plants which easily grown on this type of soil. *Hibiscus* has a tart taste, which may be the reason of its large consumption in Kwilu's region. Some authors have linked high-oxalate diets to an increased risk of kidney stones and other health problems in susceptible people [2-4]. According to Domenico et al. [4], intake of foods with high oxalate content have contributed strongly to the growing incidence of Nephrolithiasis in the last few decades, with an estimated recurrence rate higher than 50%. It has been reported that, more than 70% of most kidney stones cases are composed of calcium salts which are usually occur as calcium oxalate [4-6]. Urinary oxalate excretion is one of the risk factors that influence the development of calcium oxalate kidney stones; this excretion of oxalate in urine has a significant impact on calcium oxalate supersaturation and stone formation. High consumption of foods with high oxalate content in people having high rate of oxalate absorption (with overt intestinal disease or not) increases the excretion of urinary oxalate [7-14]. This work aimed to determine the total content of oxalate in ten species of *Hibiscus* found in Kwilu state/Democratic Republic of Congo, also to find out the variation of oxalate content according to the plant ages and after the removal of first cooking water.

2. MATERIALS AND METHODS

Ten different species of *Hibiscus* were collected in rain season, in Kwilu state (DRC), and were authenticated at the INERA Herbarium, located in the Department of Biology of the Faculty of Sciences/University of Kinshasa. The leaves of different species of *Hibiscus* were grinded with pestle and mortar separately. The oxalate's extraction was carried out according to the method described by Savage et al. [15]. An amount of 0.1 Kg of each dried *Hibiscus* specie was introduce in 4000 mL of distilled water at 100°C for 15 minutes; nitrate filter paper was used to separate soluble oxalates in aqueous solution to the residue. Total oxalates were extracted using 0.1 Kg of each *Hibiscus* specie in 4000 mL of aqueous solution of hydrochloric acid (2N) at 100°C, after 15minutes the mixture were filtrated using nitrate filter paper. Volumetric titration method described by Sarkiyayi [16] was used to determine the oxalate content in different samples of *Hibiscus* species (25 mL) using a standard solution of potassium permanganate (0.05M). The throwing of the first cooking water was done only once, as described beside. The fresh plant (0.1 Kg) was boiled at 100°C with 4000 mL of water; the boiled water was removed by filtration using nitrate filter paper; the residue was also boiled at 100°C with 4000 mL of water and filtrated to determine residual contents of total oxalates. The experiment was done in triplicate, using the same amount of *Hibiscus* plant and water at the same temperature (100 °C). Three species of *Hibiscus* (*Hibiscus acetosella Welw*, *Hibiscus* 'Red Shield'*, and Hibiscus sabdariffa var. sabdariffa)* were planted

in the experimental garden of High School of Teaching (ISP-Kikwit) in standard condition. Only three species of *Hibiscus* were selected for the study of oxalate content's variation according to the plant's age. The three species were collected in Kikwit (the capital of Kwilu state) so it was better to cultivate the three species in the experimental garden who is located at the same place where the collection of the three species of *Hibiscus* was done. Oxalate content was determined as described above, in view of being in similar conditions. No chemical fertilizers or pesticides were used; in view of mimicking the traditional practice what is done in that region, which consist to bury the leaves of plant (organic fertilizer) like *Tithonia diversifolia* (*Asteraceae*) in the soil in bed before spreading the seeds above. Our culture were done during a rain season, no supplier of water was used, like it is done in that region. The collection of the leave sample for oxalate analysis was done five times in the interval of fifteen days (15th, 30th, 45th, 60th and 90th) in triplicate in order to evaluate the differences in oxalate content according to their ages; plant collection was done by tearing or cutting a few leaves on several feet of these plants according to the sampling technique that is done in Kwilu State. The dosage of total oxalate and soluble oxalate of each age of our three species of *Hibiscus* cultivated were done separately like described previously.

3. RESULTS AND DISCUSSION

The total oxalate and soluble oxalate of ten species of *Hibiscus* used in this research are presented in the Fig. 1 with their residual contents (total oxalates) after discharging the first cooking water.

The result obtained shows that *Hibiscus acetosella* 'Panama Red' contained both the highest total oxalate content (1056 mg / 100 g of fresh weight) and highest content of soluble oxalate (865,92 mg / 100 g of fresh weight) compare to others; while *Hibiscus sabdariffa var altissima* has presented the lowest total oxalate content of 422.40 mg /100 g of fresh weight.

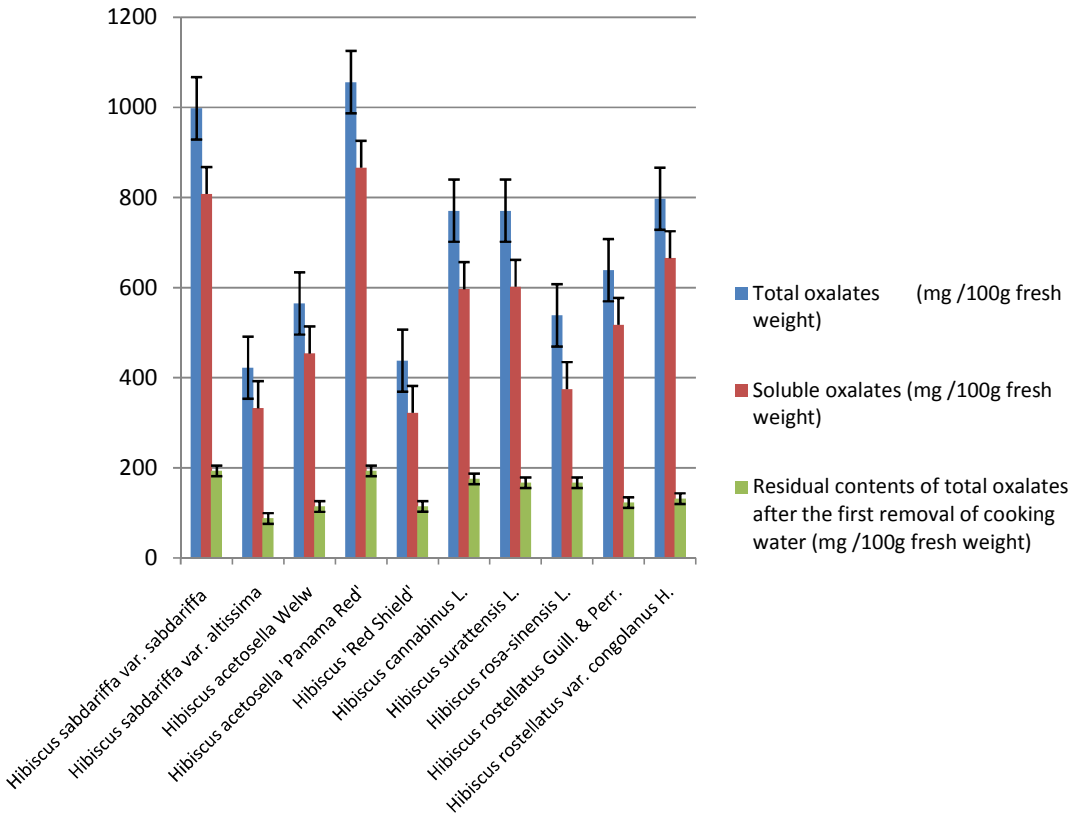

Fig. 1. The oxalate content variation

After the evaluation of oxalate content as function of the age of the plant in three randomly selected species (*Hibiscus acetosella Welw, Hibiscus* 'Red Shield'*, and Hibiscus sabdariffa var. sabdariffa*), the following results were recorded as described in the Figs. 2-4.

The results of oxalate content summarized in Fig. 1 show that there is great variability of oxalate content within different species of *Hibiscus*. This variability of oxalate content is recorded for the two varieties of *Hibiscus sabdariffa* (*H. sabdariffa* var. *sabdariffa* and *H. sabdariffa* var. *altissima*) harvested at the same place, their recorded contents are respectively 997.92 and 422.40 mg /100 g of fresh weight.

The same observation is made for the three others species (*H. acetosella* Welw, *Hibiscus acetosella* 'Panama Red' and *H.* 'Red Shield') which have respective contents of 564.96; 1056.00 and 438.2 mg / 100 g; and for the two varieties of *Hibiscus rostellatus* (*H. rostellatus Guill. & Perr. and H. rostellatus var congolanus* H.) which have oxalate contents of 638.88 and 797.28 mg / 100 g.

This variability was observed by Li and Savage, [17] in their study of oxalate content in the leaves of *Chenopodium bonus-Henricus*. They found that the broad leaves of this plant contained more oxalate (867.4 mg / 100 g) than the smaller leaves (610.5 mg / 100 g).

According to the oxalate contents found, it should be noted that *Hibiscus acetosella* 'Panama Red' contains more than all other species studied (1056 mg / 100 g fresh weight). This high content is nevertheless lower than that found by Siener et al. [18], in their study of the oxalate content in some vegetables of the families of *Polygonaceae* and *Amaranthaceae*. These researchers found a content of 1959 mg / 100 g for *Spinocia oleracea*. Guil et al. [19] recorded the content levels of oxalate ranging from 360 to 2000 mg / 100 g of *Chenopodium album*.

The results presented in Fig. 2 to 4 indicate that the oxalate content increases with plant age until it reaches a maximum of 55 to 60 days and then decreases. Oxalate is one of the compounds that plant used to protect them against herbivore; oxalate's compound may be formed during plant growth and may be decreased before getting rid of its leaves (plant old age); plant in old age may have a modified metabolism with a decrease rate of access to the plant's nutrient [20].

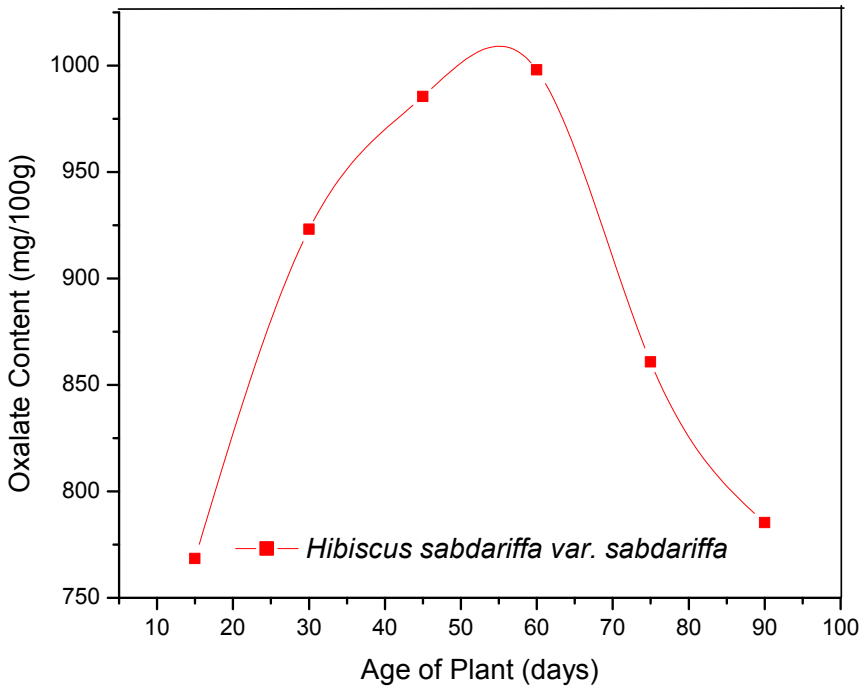

Fig. 2. Evolution of oxalate content from *Hibiscus sabdariffa var. sabdariffa*

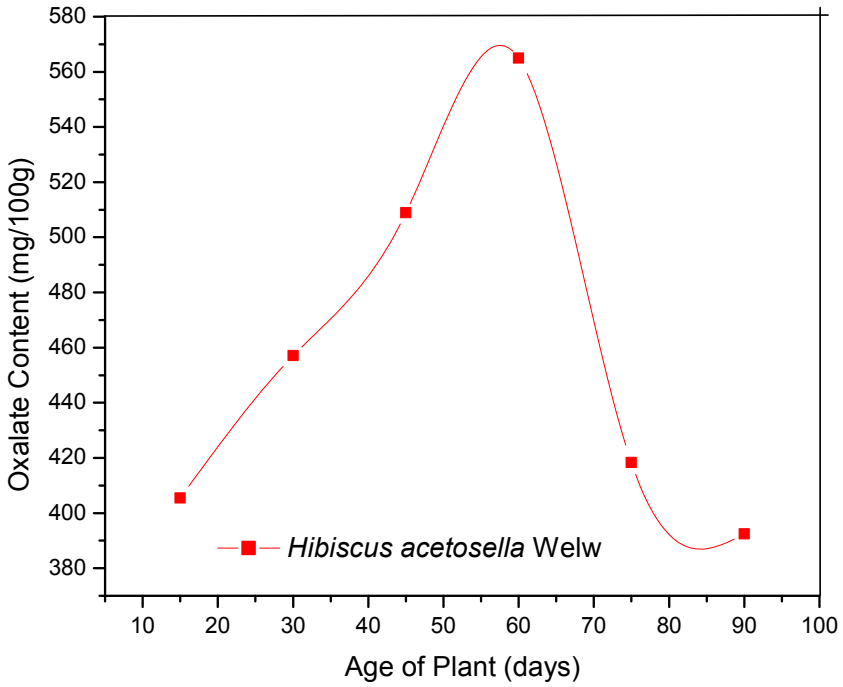

Fig. 3. Evolution of oxalate content from *Hibiscus acetosella Welw*

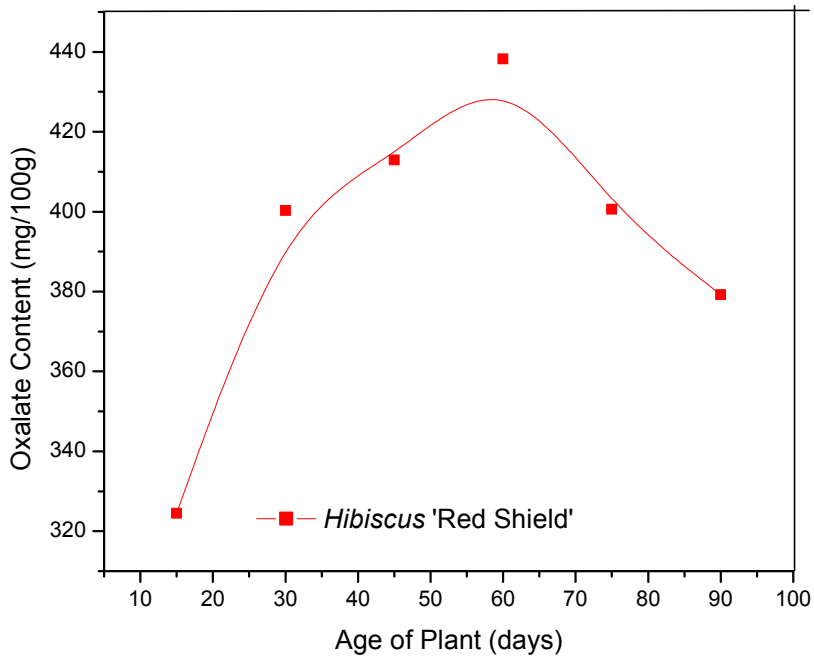

Fig. 4. Evolution of oxalate content from *Hibiscus* 'Red Shield

Oxalic acid is one of the plant's compounds that functions as part of both biochemical and physical mechanisms of defense depending upon the form of the acid present in the plant tissue.

Massey et al. [21] stated out a reduction of the risk of developing kidney stone by a restriction of high-oxalate food intake. Removal of cooking water have been used to reduce the oxalate content in some food containing high oxalate

content [22-24]. In an attempt to reduce the oxalate content in some species of *Hibiscus* which are as well consumed in Kwilu state, the first cooking water was removed. The result shows above in Fig. 1 indicate that there is a very significant reduction in this anti-nutritional factor with the total oxalate percentages eliminated ranging from 84.44% (*H. rostellatus var congolanus* H.) to 73.90% for *Hibiscus* 'Red Shield'; which may serve as protecting wall, since so many studies link the high-oxalate containing food to the risk of developing kidney stone and other health problems in susceptible people [2,4,25]. Li and savage [17] found significant differences between the oxalate levels of fresh vegetables and cooked vegetables; they reported that the fresh leaves of *Chenopodium bonus-henricus* contained 703.5 mg / 100 g of total oxalate content, while the cooked leaves contained 281.9 mg / 100 of total oxalate content.

4. CONCLUSION

The ten fresh species of *Hibiscus* used in this work showed non-negligible concentrations of oxalate, which were greatly reduced after removing the first cooking water; this may be used to avoid the risk of developing kidney stone and other health problems in susceptible people. A maximum level of oxalate concentration was found between 55 to 60 days of plant development, may suggests that the consumption of these species with less oxalate content may be done at younger or older ages.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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