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Asian Journal of Plant Science and Research, 2012, 2 (3):342-349

ISSN : 2249-7412
CODEN (USA): AJPSKY

Effect of leaf positions on the concentrations of some micronutrients, anti-nutrients and toxic substances in *Hibiscus sabdariffa* at vegetative phase

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ABSTRACT

The leaf age (which is linked to position on mother - plant) influenced the accumulation of nutrients and toxic substances by the leaves on plants. It is against this background that this study was carried out in pot experiment to examine the effect of leaf position on the levels of antinutrient (soluble and total oxalates), toxic substances (cyanide and nitrate) and some micronutrients viz; β-carotene (provitamin A), vitamin C and mineral elements (Fe, Mg, Cu, Zn, Ca Na and K) at vegetative phase of Hibiscus sabdariffa grown in nitrogen and non – nitrogen treated soil. The leaves of Hibiscus sabdariffa were harvested and analysed at three different leaf locations, namely; basal, middle and upper positions. The results obtained showed that the concentrations of cyanide and nitrate were generally higher in older leaves than younger ones. The soluble and total oxalate contents in the vegetable increased with leaf age. Vitamin C content was concentrated more in the middle leaf region compared to basal and upper leaf positions in the vegetable.The β-carotene concentration was significantly ($p < 0.05$) highest in middle leaves, closely followed by upper leaves and least in the basal leaves. Levels of Fe in the basal and middle leaves were not significantly different from each other, but the two leaf positions had significant ($p < 0.05$) higher amount of the mineral than upper leaves irrespective of the soil nitrogen levels.The concentrations Ca, Cu and Na were significantly higher in the basal leaf region compared to middle and upper leaf locations in Hibiscus sabdariffa except that no significant variation in Cu content was observed between basal and middle when nitrogen was applied. While leaf position had no significant effect on the concentrations of Mg and Zn in Hibiscus sabdariffa, the concentration of K was significantly higher in the upper leaves than the middle and basal leaves in control and nitrogen applied. The study concludes that the plant toxins are concentrated more in the older leaves than the younger ones in Hibiscus sabdariffa at vegetative phase.

Key words: *Hibiscus sabdariffa*, leaf age (leaf position), cyanide, nitrate, oxalates, micronutrients, market maturity (vegetative phase).

INTRODUCTION

Roselle (*Hibiscus sabdariffa*) belongs to the family of Malvaceace and is a popular vegetable in Indonesia, India, West Africa and many tropical regions [1, 2]. The vegetable is widely grown in the North-Eastern and middle belt regions of Nigeria [3]. In Nigeria, two botanical varieties were recognised, the red variety in which the calyx is used for the preparation of "sobo" drink and the green variety which calyx and leaves are used in stew and sauces [4, 5, 6, 7]. This plant has been found to thrive on a wide range of soil conditions. It can perform satisfactorily on relatively infertile soils but for economic purposes, a soil well supplied with organic materials and essential nutrients is important in the productions [1, 6]. It can tolerate relatively high temperature throughout the growing and fruiting periods. The plant requires an optimum rainfall of approximately 45 – 50 cm distributed over a 90 - 120 day growing period [1]. The leaves and calyx of the green variety are very rich in vitamin C, β-carotene and riboflavin with some major mineral elements [8]. The leaves of *Hibiscus sabdariffa* also contain phytic acid, tannin, oxalate, nitrate and glucoside such as delphinidin-3-monoglucosides and delphinidin which are toxic to animal and human tissue at high concentration [9, 10, 11]. Tannins form complexes with protein [12]. Phytic acid chelates minerals and form complexes with proteins, and thereby affects their nutritive values [13]. Cyanogenic glucosides also found in this plant are inhibitors of cytochrome oxidase enzyme [14, 15]. The concentrations of these nutrients and toxic substances in the leaves of the *Hibiscus subdariffa* like other leafy vegetable are known to be influenced by the position of the leaf on the mother – plant. It is for this reason that this research was designed to investigate the effect of three leaf positions, namely; basal, middle and upper leaf locations on the levels of some micronutrients, antinutirients and toxic substances in the leaves of *Hibiscus subdariffa.* This is aiming at determing the leaf position that will provide higher nutrient concentrations with lower levels of phytotoxins in *Hibiscus subdariffa*.

MATERIALS AND METHODS

The study area

The pot experiment was conducted in the nursery of the School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State of Nigeria.

Niger State has a Savannah climate characterized by maritime. The geographical location of Minna is at longitude 9^o 40'N and latitude 6^o 30'E. Minna lies in the Southern Guinea Savannah zone of Nigeria and has a sub - humid semi arid tropical climate. The raining season is between April and October. About 90% of the total rainfall occurs between the month of June and September. The mean annual rainfall is in the range of 1200 – 1300 mm. The temperature of this zone rarely falls below 22^oC with peaks temperature of 40^oC in February /March and 30^oC in November /December. Wet season average temperature is about 29^0C. The Dry season occurs between November and March while harmattan which is chacterised by dry air is between November and February [16].

Soil sampling and analysis

The soil used in this study was collected from Minna. The soil has been classified as Inseptisol [17]. The bulked sample was collected during the dry season from the field which has been under fallows for about four years. The bulked soil sample was passed through 2 mm sieve. Sub-sample of the soil was subjected to routine soil analysis using procedure described by [18]. The soil particle sizes were analyzed using hydrometer method; pH was determined potentiometrically in the water and 0.01M $CaCl_2$ solution in a 1: 2 soil/ liquid using a glass electrode pH meter and organic carbon by Walkey-Black method [18]. Exchange acidity (E.A H^+ and Al^{3+}) was determined by titration method [18]. Exchangeable Ca, Mg, K and Na were leached from the soil sample with neutral 1N NH_4OAc solution. Sodium and potassium were determined by flame emission spectrophotometry while Mg and Ca were determined by E.D.T.A versenate titration method [18]. Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No 1 method [18].

Sources of seeds

The seeds of roselle (*Hibiscus sabdariffa*) were obtained from Schools of Agriculture and Agricultural Technology's Farm/Nursery of Federal University of Technology, Minna.

Planting, experimental design and nursery management

About ten seeds of *Hibiscus sabdariffa* were planted in a polythene bag filled with 10.00 kg of top soil and after emergence the seedlings were thinned to two plants per pot. The factorial design was adopted to determine the effect three leaf age/positions (basal, middle and upper locations) in control and nitrogen treated vegetable. Each treatment had 10 pots replicated three times. This gave a total of 60 pots for the vegetable. The seedlings were watered twice daily (morning and evening) using watering can and weeded regularly. The experimental area and the surroundings were kept clean to prevent harbouring of pest. The pots were lifted from time to time to prevent the roots of the plants from growing out of the container. Insects were controlled using Sherpa plus (Saro Agro Sciences) four weeks after planting at the rate of 5 ml per 5 litres of water.

Fertilizer treatment

The fertilizer treatments for *Hibiscus sabdariffa* are stated below:
F_1 (control): 0 N, 40 mg P_2O_5/kg soil and 40 mg K_2O/kg soil
F_2: 40 mgN/kg soil, 40 mg P_2O_5/kg soil and 40 mg K_2O/kg soil

Harvesting

The leaves of *Hibiscus sabdariffa* grown in pot experiment in control and nitrogen fertilized soil were harvested at three different locations on the plant (basal, middle and upper locations) at the vegetative phase of the plant development.

Sample analysis

The soluble and total oxalates concentration in the leaves of *Hibiscus sabdariffa* obtained from the three different leaf positions were determined by titrimetric method of [18]. Nitrate content in the test samples was determined by the colourimetric method [20]. Alkaline picrate method was used to analyse the cyanide concentration [21]. The mineral elements (Fe, Mg, Zn, Cu, Ca, Na and K) in samples were determined according to the method of [22]. The ascorbic acid concentration was determined by 2, 6-dichlorophenol indophenols method [23] while the estimation of β-carotene was done by ethanol and petroleum ether extraction method [24].

Statistical analysis

Analysis of variance (ANOVA) was carried out using statistical package Minitab to determine variation between three levels of age of plant leaves. The DUNCAN's Multiple Range Test (DMRT) was used for comparison of means.

RESULTS

Physical and Chemical Properties of Soil

Result of analysis of the soil used for pot experiment is presented in Table 1. The texture class of the soil is sandy loam signifying that the water holding capacity is moderate. The organic matter content, total nitrogen and available phosphorus are low. Sodium and calcium contents are moderate while magnesium and potassium contents are high. The CEC (cation exchange capacity) is moderate while base saturation percentage is high. Soil pH shows that the soil is slightly acidic.

Table 1 Some Physical and Chemical Properties of the Soil (0 – 20cm) Used for Pot Experiment

Parameters	Values
Sand (%)	71.40
Silt (%)	18.00
Clay (%)	7.60
pH (in H_2O)	6.54
pH (in 0.1M C_aCI_2)	5.25
Organic Carbon (%)	0.83
Organic Matter (%)	1.43
Total nitrogen (%)	0.05
Available phosphorus (mg/kg)	6.64
K (cmol/kg)	0.92
Na (cmol/kg)	0.68
Mg (cmol/kg)	4.80
Ca (cmol/kg)	8.00
E. A (H^+ +AL^{3+})(cmol/kg)	1.50
CEC (cmol/kg)	15.90
Base saturation (%)	90.55
Texture class	sandy loam

**Values represent means of triplicate determinations.*

Effect of leaf position on antinutrients and vitamins concentration

The determination of effect of leaf position on antinutrients and vitamins concentration in *Hibiscus sabdaliffa* showed that no significant differences in cyanide concentration was observed between basal (522.89 ± 30.42 mg/kg) and middle (472.57 ± 6.83 mg/kg) leaves, however, the two leaf positions each was significantly ($p < 0.05$) higher in cyanide concentration than upper (383.45 ± 25.41 mg/kg) region in control. When the plant received nitrogen fertilizer, the same trend of result was obtained, with no significant variations in cyanide concentration between basal (466.81 ± 75.17 mg/kg) and middle (428.92 ± 36.80 mg/kg) leaves , but upper leaves (362.84 ± 33.28 mg/kg) had significant ($p < 0.05$) concentration of the compound than leaves obtained each from the two leaf locations (Table 2).

Results obtained from the analysis of nitrate showed that basal leaves (179.22 ± 12.73 mg/kg) had the highest significant ($p < 0.05$) amount of nitrate, followed by middle (37.72 ± 8.99 mg/kg) and lowest in upper (17.79 ± 8.59 mg/kg) leaves in control vegetable. Similarly with application of nitrogen fertilizer, basal leaves were highest in in nitrate concentration (205.55 ± 4.81 mg/kg), followed by middle leaves (57.22 ± 8.83 mg/kg) and least in the upper (42.78 ± 16.55.76 mg/kg) leaf positions.

The determination of soluble oxalate concentration in the vegetable showed that leaf positions had no significant ($p > 0.05$) effect on the antinutrient concentration in the leaves of *Hibiscus sabdaliffa* in control. However, with the application of nitrogen fertilizer, though no significant variation in soluble oxalate concentration was observed between basal (1.53 ± 0.06 g/100g) and middle (1.45 ± 0.11 g/100g) leaves, the antinutrient content in the two leaf locations were significantly ($p < 0.05$) higher than in the upper leaves (1.22 ± 0.05 g/100g). The total oxalate concentrations in the middle leaves was not significantly ($p > 0.05$) different from upper leaves, however, basal leaves had significant ($p < 0.05$) higher concentration of compound than in the two leaf positions in both control and nitrogen treated *Hibiscus sabdaliffa* (Table 2).

The significant ($p < 0.05$) increased in β-carotene concentration observed in the three leaf positions in *Hibiscus sabdaliffa* irrespective of soil nitrogen levels is in the following order Middle > Upper > Basal leaves (Table 2).

Analysis of vitamin C conconcentration in *Hibiscus sabdaliffa* showed that no significant variations in the vitamin content was observed between basal and upper leaves, but the two leaf positions were significantly ($p < 0.05$) lower in the vitamin concentration than in the middle leaves in control and nitrogen treated. The mean values for basal leaves in control (10.45 ± 0.99 mg/100g) and nitrogen applied vegetable (12.42 ± 1.49 mg/100g) were not significantly different from the levels in upper leaves (10.45 ± 0.99 mg/100g and 11.03 ± 0.99 mg/100g respectively). The mean value of the vitamin in the middle leaves for control (16.61 ± 2.20 mg/100g) and nitrogen applied (18.19 ± 3.47 mg/100g) were significantly ($p < 0.05$) higher than the two leaf positions (Table 2).

Table 2. Effect of leaf position on antinutrients and vitamins concentration in *Hibiscus sabdaliffa* at vegetative phase

Antinutrients and vitamins	Leaf positions		
	Basal leaves	Middle leaves	Upper leaves
Cyanide (mg/kg DW), Control	522.89 ± 30.42^{b}	472.57 ± 6.83^{b}	383.45 ± 25.41^{a}
Cyanide (mg/kg DW). Nitrogen applied	466.81 ± 75.17^{b}	428.92 ± 36.80^{b}	362.84 ± 33.28^{a}
Nitrate (mg/kg DW), Control	179.22 ± 12.73^{c}	37.72 ± 8.99^{b}	17.79 ± 8.59^{a}
Nitrate (mg/kg DW), Nitrogen applied	205.55 ± 4.81^{c}	57.22 ± 8.83^{b}	42.78 ± 16.55^{a}
Soluble oxalate (g/100g DW), Control	1.70 ± 0.09^{a}	1.63 ± 0.07^{a}	1.54 ± 0.17^{a}
Soluble oxalate (g/100g DW), Nitrogen applied	1.53 ± 0.06^{b}	1.45 ± 0.11^{b}	1.22 ± 0.05^{a}
Total oxalate (g/100g DW), Control	2.33 ± 0.14^{b}	1.97 ± 0.09^{a}	1.93 ± 0.08^{a}
Total oxalate (g/100g DW), Nitrogen applied	2.04 ± 0.60^{b}	1.88 ± 0.07^{a}	1.85 ± 0.09^{a}
β-carotene (μg/100g FW), Control	3706.00 ± 217.30^{a}	6518.00 ± 80.00^{c}	5989.00 ± 89.90^{b}
β-carotene (μg/100g FW), Nitrogen applied	6105.00 ± 149.00^{a}	7874.30 ± 155.90^{c}	7220.00 ± 89.60^{b}
Vitamin C (mg/100g FW), Control	10.45 ± 0.99^{a}	16.61 ± 2.20^{b}	10.45 ± 0.99^{a}
Vitamin C (mg/100g FW), Nitrogen applied	12.42 ± 1.49^{a}	18.19 ± 3.47^{b}	11.03 ± 0.99^{a}

DW = Dry weight, FW = Fresh weight, Control = No nitrogen applied. Values represent means of triplicate determinations. Mean values carrying the same superscripts across rows do not differ significantly from each other ($P > 0.05$).

Effect of leaf position on mineral elements concentration

The studies conducted on the effect of leaf positions on mineral element concentrations in *Hibiscus sabdariffa* showed that there was no significant differences in the Fe concentration between basal (35.90 ± 5.30 mg/kg) and middle (34.50 ± 1.42 mg/kg) leaves, but the two leaf positions had significant ($p < 0.05$) higher amount of the mineral than upper leaves (29.10 ± 4.99 mg/kg) in the control vegetable. When the plant received nitrogen fertilizer, the same trend of result was obtained with concentration of Fe in basal, middle and upper leaves of 39.60 ± 8.81 mg/kg, 31.90 ± 6.61 mg/kg and 23.80 ± 1.90 mg/kg, respectively (Table 3).

Results obtained from the determination of Mg and Zn indicated that leaf positions had no significant effect on these mineral elements content in the leaves of *Hibiscus sabdariffa* irrespective of the nitrogen fertilizer levels (Table 3).
The concentration of Cu in the middle leaves (1.83 ± 0.20 mg/kg) was not significantly different from upper leaves (1.56 ± 0.15 mg/kg); however, both leaf positions were significantly ($p < 0.05$) lower in the mineral content than the

basal leaf (3.02 ± 0.03 mg/kg) in the control. When nitrogen fertilizer was applied, no significant variation in Cu conconcentration was recorded between basal (3.40 ± 0.29mg/kg) and middle (3.29 ± 0.96mg/kg) leaves; the two leaf positions were significantly ($p < 0.05$) higher in the mineral content than the upper (1.99 ± 0.24mg/kg) leaf region (Table 3).

The determination of Na in the vegetable showed that there was no significant variation in the mineral content between middle and upper leaves; however, basal leaves had significant higher Na concentration than the two leaf positions in control and nitrogen fertilized *Hibiscus sabdariffa* (Table 3).

The analysis of Ca revealed that the concentration of the mineral in the middle leaves was not significantly ($p > 0.05$) different from upper leaves, but basal leaves had significant ($p < 0.05$) higher concentration of Ca than middle and upper leaves in both control and nitrogen treated *Hibiscus sabdaliffa*. The mean values for middle leaves in control (22.11 ± 1.23 mg/kg) and nitrogen applied (23.82 ± 3.79 mg/kg) were not significantly different from the levels in upper leaves (22.89 ± 3.59 mg/kg and 23.64 ± 1.36 mg/kg respectively). The mean values of the mineral in the basal leaves for control (46.18 ± 5.88 mg/kg) and nitrogen applied (45.87 ± 0.05 mg/kg) were significantly ($p < 0.05$) elevated than the middle and upper leaves (Table 3).

Result obtained from the investigation of K content in the three leaf locations showed that there were no significant differences in the mineral element concentration between basal and middle leaves, however, its level in the upper leaves was significantly ($p < 0.05$) higher than basal and middle leaves in both control and nitrogen treated *Hibiscus sabdariffa*. The mean values for basal leaves in control (33.08 ± 6.74 mg/kg) and nitrogen applied vegetable (36.98 ± 3.37 mg/kg) were not significantly different from the levels in middle leaves (38.28 ± 3.00 mg/kg and 38.09 ± 2.90 mg/kg respectively). The mean value of the mineral in the upper leaves for control (46.18 ± 5.88 mg/kg) and nitrogen applied (45.87 ± 0.05 mg/kg) were significantly ($p < 0.05$) higher than the two leaf positions (Table 3).

Table 3. Effect of leaf position on mineral concentration in *Hibiscus sabdaliffa* at vegetative phase

Minerals	Leaf positions		
	Basal leaves	Middle leaves	Upper leaves
Fe (mg/kg), Control	35.90 ± 5.30^b	34.50 ± 1.42^b	29.10 ± 4.99^a
Fe (mg/kg) , Nitrogen applied	39.60 ± 8.81^b	31.90 ± 6.61^b	23.80 ± 1.90^a
Mg (mg/kg), Control	17.87 ± 0.59^a	18.41 ± 0.97^a	19.92 ± 10.80^a
Mg (mg/kg), Nitrogen applied	19.09 ± 0.39^a	20.02 ± 0.71^a	23.27 ± 5.21^a
Zn (mg/kg), Control	0.04 ± 0.02^a	0.03 ± 0.01^a	0.03 ± 0.01^a
Zn (mg/kg), Nitrogen applied	0.03 ± 0.01^a	0.02 ± 0.01^a	0.02 ± 0.01^a
Cu (mg/kg), Control	3.02 ± 0.03^b	1.83 ± 0.20^a	1.56 ± 0.15^a
Cu (mg/kg), Nitrogen applied	3.40 ± 0.29^b	3.29 ± 0.96^b	1.99 ± 0.24^a
Ca (mg/kg), Control	25.42 ± 3.89^b	22.11 ± 1.23^a	22.89 ± 3.59^a
Ca (mg/kg), Nitrogen applied	26.96 ± 0.50^b	23.82 ± 3.79^a	23.64 ± 1.36^a
Na (mg/kg), Control	3.38 ± 0.42^b	2.54 ± 0.37^a	2.48 ± 0.33^a
Na (mg/kg), Nitrogen applied	3.38 ± 0.21^b	2.96 ± 0.04^a	2.86 ± 0.64^a
K (mg/kg), Control	33.08 ± 6.74^a	38.28 ± 3.00^a	46.18 ± 5.88^b
K (mg/kg), Nitrogen applied	36.98 ± 3.37^a	38.09 ± 2.93^a	45.87 ± 0.05^b

Control = No nitrogen applied. Values represent means of triplicate determinations. Mean values carrying the same superscripts across rows do not differ significantly from each other ($P > 0.05$).

DISCUSION

The significantly higher cyanide concentrations in the basal than upper leaves in *Hibiscus sabdariffa* is in harmony with the report of [25, 26, 27], who have reported that the cyanide concentrations increased with age in cassava leaves, crucifers and *Telfairia occidentalis*, respectively. The reason for higher level of cyanide in basal leaves than the upper leaves could be that the enzymes responsible for synthesis of the cyanide may be more active in fully developed leaves where the metabolic activities are at maximum than the immature leaves. These results however, disagree with the report of [28, 29], who observed that the level of this respiratory poison is concentrated in younger leaves of sorghum than the older leaves. The observed variations in the cyanide concentration in the different leaf position of this vegetable from those of the previous work reported by different authors may be due to differences in cultivars and environmental factors.

The significantly higher nitrate content in basal leaves followed by middle and least in the upper leaves in *Hibiscus sabdariffa* may indicate that nitrate content in leaves of the vegetables increased with leaf age. This result is in line with the report of [30] who attributed the higher accumulation of nitrate concentration in older leaves than younger ones to lower activity of nitrate reductase enzyme in the former than in the later. This enzyme is responsible for the reduction of nitrogen to amino acid used for protein synthesis. Low activity of the enzyme in the basal leaves reduce the rate of protein formation from nitrogen and this may favours the accumulation of nitrogen and its subsequent oxidation to nitrate in the affected leaf regions. This finding is likely to be correct since the same author had also reported that nitrate had a significant negative correlation with nitrate reductase activity. In general, the higher and lower nitrate concentrations observed in the different leaf positions may be due to the lower and higher nitrate reductase activity respectively, in those leaf regions.

The generally highest level of soluble and total oxalates in basal leaves followed by middle and least in the upper leaf region in *Hibiscus sabdariffa* are in harmony with the submission of [27, 31] that the oxalates (soluble and total) were higher in older leaves than younger ones in *Telfairia occidentalis* and *Spinacia oleracea*, respectively. The reason for this observation could be that the older leaves are fully matured with optimum metabolic activity leading to the production of oxalates. The results however, disagree with the finding of [32, 33]. These authors independently observed that oxalate concentrations in younger leaves were slightly higher than in the older leaves in *Diplazium sammatil* and *Colocasia esculenta*, respectively. Our present study clearly revealed that the levels of these phytotoxins are concentrated more in the older leaves of *Hibiscus sabdariffa* than the younger ones. Thus consumption of the younger leaves of the vegetable which are located in the upper leaf region on the mother - plant is likely to reduce the public health problems linked with higher intake of the antinutrients and toxic substances in the vegetable.

The observed higher concentrations of β-carotene and vitamin C in the middle leaves than basal and upper leaves of *Hibiscus sabdariffa* agrees with the report of [34] which show that these micronutrients are concentrated in the middle leaves of *Amaranthus cruentus* than basal and upper leaves. This higher levels of the provitamin A and vitamin C in middle leaves than the basal and upper leaves in *Hibiscus sabdariffa* could be an indication that the leaves in the middle region of the vegetable are fully matured with optimum physiological and metabolic activities leading to the production of these compounds. Whereas in the in basal leaves, though they are fully matured, the leaves in this region are withdrawing some oxidizable nutrients due to aging [35]. Wilting which occurs in older lower leaves may be another reason responsible for the lower level of these micronutrients in basal leaves compared to middle leaves. This observation may be correct because wilting decreases the vitamin C content [36, 37, 38]. Leaves in the upper region of *Hibiscus sabdariffa* are still developing and are immature with low physiological and metabolic activities leading to the formation of β-carotene and vitamin C. The higher concentrations of the water soluble vitamin and provitamin A in middle leaves of the vegetable does have not much nutritional advantage over the upper leaves. This is because the concentration of β-carotene in the upper leaves can still provide enough of vitamin A to meet the adult recommended daily allowance of 900 µg [39, 40]. While the concentration of vitamin C in the two leaf regions of *Hibiscus sabdariffa* was lower than the recommended daily allowance of 60 mg [38, 40] if 100 g of the leaves of the vegetables are consumed. Thus considering the crucial functions of this water soluble vitamin, such as anti-infective properties, promoting wound healing, acting as antioxidant and boosting of immune system, supplementation of the vitamin from other sources (such as from pharmaceutical or fruits) in either cases will be necessary.

The significant higher concentrations of Fe, Cu and Ca in older leaves than the younger leaves concur with the findings of [32, 35, 41] who reported that these mineral elements in plants are generally higher in older leaves than the younger ones. [35, 41], attributed the higher level of these minerals in the older leaves compared to the younger leaves due to their immobile nature in plants. The low mobility of Fe in the plant is probably due to its precipitation in the older leaves as insoluble oxides or phosphates or to the formation of complexes with phytoferritin, an iron-binding protein found in the leaf and other plant parts [35, 42]. Although the concentrations of Fe and Cu in the leaves obtained from upper leaf position in *Hibiscus sabdariffa* was lower than other leaf locations, the level of these trace elements in the upper leaves could provide the body with the recommended daily allowance of 18 mg/day for Fe and 1.5 – 3.0 mg/day for Cu [43]. Thus this high level of these mineral elements in the basal leaves has no nutritional benefit over the leaves obtained from middle and upper leaf regions. Similarly, the concentrations of Ca in each of the three leaf positions are lower than recommended daily allowance of 800 mg/day [40], therefore supplementation of the mineral from other sources such as fruits and pharmaceuticals becomes necessary for all the leaves from the three leaf locations.

The significantly higher concentration of K in upper leaves than basal and middle leaves in *Hibiscus sabdariffa*, justify the highly mobile nature of this mineral that play an important role in regulation of the osmotic potential of plant cell [35]. Since K can be mobilized readily to younger leaves, the concentration appears to be higher in the upper leaf region than the basal leaf position [35, 41].

CONCLUSION

Our current study divulges that the antinutrients and toxic substances in *Hibiscus sabdariffa* are concentrated more in the older leaves than the younger ones. It therefore means that inclusion of younger leaves (especially those obtained from upper leaf region) of *Hibiscus sabdariffa* in our meals will reduce the health problems connected with high intake of cyanide (respiratory poison), nitrate (cancer and metheamoglobinemia) and oxalates (kidney stone) and still provide micronutrients in amount to meet our nutritional requirements. This practice will go along way in recuperating the general wellbeing of individual and the general public.

REFERENCES

[1] H.D. Tindal; Vegetable in the tropics. Macmillan Edu. Ltd. Hampshire, **1986**. pp 267 – 268.
[2] F.E.F. Babatunde, *Afri. Crop Sci. J.*, **2003**, 11 (1): 43 – 47.
[3] H.O. Akanya, S.B. Oyeleke, A.A. Jigam, F.F. Lawal, *Nig. J. Biochem.*, **1997**,12: 77 – 79.
[4] Y.A. Duke; Hand book of Medicinal herbs. Living stone Group Ltd, Edinburgh. **1997**.
[5] D. Syndenham; Success in vegetable production. Macmillam London, **1985**.
[6] I.G. Adanlawo, V.A. Ajibade; *Pak. J. Nutr.*, **2006**, 5 (6): 555 – 557.
[7] A. Ojokoh; *Pak. J. Nutr.*, **2006**, 5 (2): 110 – 113.
[8] S.O.Babalola; Chemical analysis of roselle leaf (*Hibiscus Sabdariffa*), In Proceeding of 24th annual conference of NIFST, **2000**, 228 – 229.
[9] J. Morton; Roselle in fruits of warm climate Juliamiami, Florida., **1987**, 281 – 286.
[10] V.O. Ojokoh, F.A. Adetuye, E. Akinyosoye, O. Oyetayo, *J. Food Technol. Afr.*, **2002**, 7: 75 – 78.
[11] A. Musa, E.O. Ogbadoyi, J.A.Oladiran, *Int. J. Appl. Agric. Res*., **2012**; 7(1):27 – 36.
[12] L. Goldstein, T. Swain, *Phytochemical*., **1963**, 2: 271 – 283.
[13] R. J. Evans, S.L.Badndemer, J. Agric. Food Chem., **1967**, 15: 439 – 443.
[14] V.A.Aletor, *Vet. Human Toxicol.*, **1993**, 35: 57 – 67.
[15] A.Musa, E.O.Ogbadoyi, Asian J. Bioch., **2012**; 7(2): 63 -79.
[16] A.O.Osunde, A. Alkassoum, Agro. *Africaine Numero Special*., **1998**,1: 361 – 373.
[17] FDALR, The reconnaissance soil survey of Niger State. Soil report. Federal Department of Agricultural Land Resources, **1985**, 195.
[18] A.S.R. Juo, Selected methods of soils and plants analysis: Farming systems program – Manual series No. 1. Ibadan, IITA. **1979**, 3 – 15.
[19] L.O.Oke, *J. Am. Dietetic. Assoc*., **1966**, 53: 130 – 132.
[20] A.M.K. Sjoberg, T.A. Alanka, *J. AOAC Int.*, **1994**, 77 (2): 425 – 430.
[21] C.O. Ikediobi, G.O.C.Onyia, C.E. Eluwah, *Agric. Biol. Chem.*, 44: 2803 – 2808.
[22] F.C.Ezeonu, A.Musa, C.D.Stanly, C.E.Oswald, *Environmentalist.*, 200, 22; 237-240.
[23] T.Eleri, R.E.Hughes, *Phytochemistry.* **1983**, 2 (11): 2493.
[24] A.Musa, M.I.S.Ezenwa, J.A.Oladiran, H.O.Akanya, E.O.Ogbadoyi, *Afr. J. Agric. Res*., 5 (22): 3075 – 3081.
[25] Cleveland, D.A. and Soleri, D (**1991**). Food from dry land garden: An ecological, nutritional and social approach to small scale house food production. Publ. Centre for People. Food and Environment (CPFE) USA. **1991**, 26 – 28.
[26] Carmen, W., Angelita, D.S. and Henrique, V. P (**2007**). Antinutrients in the cassava (*Manihot esculenta crantz*) leaf powder at three ages of the plant. Ciencia e Technologia de Alimentos. http;//www.Scielo.br/Scielo.php? Script = Sci- arttext & pid = S 0101- 20612007000100019 & Ing = e ---
[27] A.Musa, E.O.Ogbadoyi, *Am. J. Expt. Agric*., **2012**; 2(2): 219 – 232.
[28] D.W. Richard, Cooperative Extension Service: Cooperative Extension work acts may 8 and June 30, **1914**, as amended, Kansas State University, County Extension Councils. Extension Districts and U.S. Department of Agriculture Cooperating, **1991**
[29] D.C.Rodney, M.D.Elba, *J. Sci. Food Agric*., **2006**, 33 (3): 269 – 275.
[30] S.U.Anjana, I.Muhammed, Y.P.Abrol, *Current Sci.*, **2007**, 92(3): 355 – 360.
[31] G.H.Beis, A.S.Simos, C.C.Dogras, *Int. Soc. Hort. Sci.*, **2007**

http;//www.actahort.org/members/showpdf?Booknrarnr=579_115.
[32] M.E.Bassey, U.I.Etuk, M.M.Ibe, B.A. Ndon, *Plant Foods for Human Nutr*. **2004**, 56 (1): 7 – 12.
[33] K.V.Oscarsson, G.P.Savarge, *Food Chem.*, **2007**, 101 (2): 559 – 562.
[34] A.Musa, J.A.Oladiran, M.I.S.Ezenwa, H.O.Akanya, E.O.Ogbadoyi, Afr. J. Biotechnol., **2011**; 10(48): 9857 – 9863.
[35] L.Taiz, E. Zeiger; Plant physiology. Sinauer Associates, Inc. Sunderland, Massachusetts, **2002**.
[36] M.Fafunso, O. Bassir, *Food chem*., **1976**, 2: 51 – 55.
[37] O.O.Keshinro, A.O. Ketiku, *Food Chem.*, **1983**, 3: 303 -309.
[38] O.Olaofe, *Nig. J. Nutr. Sci.*, **1992**, 13(1 and 2): 1 – 7.
[39] H.O. Akanya; Retinol: The vitamin of life. Federal University of Technology, Minna, Innuagural lecture series, No. 5, Scan Prints Nig. Ltd, **2004**,
[40] D.P.R.George; Newlife style: Enjoy it. Editorial Safeliz, Spain, **1999**.
[41] G.Hochmuth, D.Maynard, C.Vavrina, E.Hanlon, E.Simonne, Plant tissue analysis and interpretation for vegetable crops in Florida: University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS). **2004**, 1 – 79.
[42] S.H.Oh, S.W. Cho, T.H. Kwon, M.S. Yang, *J. Bioch. Mol. Biol.*, **1996**, 29: 540 – 544.
[43] N.W. Tietz, A.B. Carl, R.A.Edward; Tietz test book of clinical Chemistry, W.B. Saunders Company, London, **1994**.