



Comparative Studies of Trace Metals Determination of Three Species in Calyces of *Hibiscus sabdariffa* Linn

Abdulazeez¹ A., Ibrahim¹ I.L., Abdullahi² U., Abubakar³ M. and Uthman⁴ A.

¹Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria

²Department of Chemistry, Nasarawa State University, Keffi, Nigeria

³Department of Chemistry, Kaduna State University, Kaduna, Kaduna State, Nigeria

⁴Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

ABSTRACT

Hibiscus sabdariffa Linn belong to the family malvaceae. It is an annual herbaceous sub- shrub with more than 300 species which are distributed in tropical and sub-tropical regions around the world. Most hibiscus species are used as ornamental plants, medicinal values; among them is *Hibiscus sabdariffa* commonly named as 'Red sorrel' and 'Roselle'. *Hibiscus sabdariffa* (calyces) have been found to be rich in vitamin, antioxidant and minerals. Therefore, the aim of this work is to determine the trace metals of this plant and to make a comparison. Three samples of calyces were collected, identified as light green, bright red and dark red. They were washed with distilled water and air dried for two weeks and grounded to uniform powder using wood type of mortar and pestle. The trace metal was carried out by using 5.0g of sample in the powdered form was accurately weighed and digested in (5:1) mixture of nitric acid and perchloric acid. After digestion, few drops of concentrated HCl was added. The solution was heated gently and then filtered. The entire filtrate was diluted up to 100cm³ with distilled water. The filtrate solution was used for analysis of elements of interest by AAS using suitable hollow cathode lamps. The mineral presence were essential and non essential heavy metals such as Fe, Zn, Cu and Pb. Iron had the highest contents with ranged between (4.526 – 5.150)mg/kg, Zn (0.044 – 0.048)mg/kg, Cu (0.129 – 0.089)mg/kg and Pb not detected. The levels were within WHO acceptable limits. The light green, bright red and dark red calyces of *Hibiscus Sabdariffa* L. contain nutrients presence give credence of its medicinal values.

Keywords: Trace metal composition, Distillation, Uniform powder, Digestion

INTRODUCTION

The contribution of medicinal plants to sciences in the traditional system of medicine for curing diseases has been interestingly on the increased and consumer demand have promoted the development of herbal products as dietary supplements. In view of renewed interest, herbal medicines have a prominent role to play in the pharmaceutical and health markets of the 21st century. It has been reported that whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals. Both the deficiency and excess of essential micronutrients and trace toxic metals may cause serious effects on human health (Singh and Sharma, 2010). The use of medicinal plants as therapeutics and dietary supplements goes back beyond history, but has increased substantially in the last decade. However, the safety of their use has recently been

questioned due to the reports of illness and fatalities (Singh and Sharma, 2010). WHO (1998) reported that medicinal plants which form the raw materials from the finished products may be checked for the presence of heavy metals and further, regulates maximum permissible limits of toxic metals like lead and arsenic which account to about 1.0 - 10 mg/L. Medicinal herbs are easily contaminated during growth, development and processes. After collection and transformation into dosage form, the heavy metals confined in plants finally enter the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pains, skin eruptions, intestinal ulcer and different types

Received 23 November, 2018

Accepted 17 December, 2018

Address Correspondence to:

aabdulazeez@ibbu.edu.ng

of cancers (Singh and Sharma, 2010). Plant constituents, as the word implies, are the individual chemicals from which plants are made. These constituents are organic in nature and synthesized in plants by the activity of individual cells. The process by which these complex organic chemical constituents are formed, utilizes simple substances like enzymes by biosynthesis (Ahmed, 2007).

Plants and their products have formed the basis of medicines essentially. Several compounds, which are pharmaceutically and medicinally important, are derived from plant sources. However, the medicinal value depends on the nature of its constituents. Active constituents are those chemical substances which are solely responsible for therapeutic activity of plants. A large number of theories have been proposed as to why these compounds are formed in plants. It is likely that many of them are synthesized as part of chemical defense mechanism to protect the plant. On the other hand, chemical constituents present in plants that do not possess any definite therapeutic value are known as inactive constituents. The formation of the various active and inactive constituents of plants involves various metabolic pathways. Hence, the inactive plant constituents are termed primary plant metabolites, whereas the active plant constituents are termed secondary plant metabolites (Ahmed, 2007). Primary plant metabolites are simple polymeric molecules that contain high-energy bonds although do not possess therapeutic activity but are essential for the life processes of plants. These are used for the biosynthesis of secondary metabolites like the Carbohydrates, proteins, lipids and nucleic acids (Ahmed, 2007).

Secondary metabolites on the other hand, are complex organic molecules biosynthesized from primary metabolites in plant cells. They are unique to a plant or group of plants, generally possess therapeutic activity, neither essential for plants life nor contain high energy bonds. These are usually stored in vacuoles. Secondary metabolites are classified as: alkaloids, glycosides, tannins, phenolic compounds, volatile oils, terpenoids, saponins, steroids, resins and bitter constituents. These are used as medicines, food, flavours, colours, dyes, poisons and perfumes. It is estimated that 1/4th of prescribed drugs contains at least

one chemical originally identified from plants (Ahmed, 2007).

Toward the end of the 20th century, epidemiological studies and associated meta-analyses suggested strongly that long-term consumption of diets rich in plant foods offered some protection against chronic diseases, especially cancer (Wallstrom *et al.*, 2000). This is attributed to uncontrolled production of free radicals that was thought to be significantly implicated in the treatment of cancer (Guyton and Kensler, 1993). These observations focused attention on the possible role of radical scavenging and radical suppressing nutrients and non-nutrients in explaining the apparent benefit of such diets (Weinberger, 1991).

Roselle is an *Hibiscus sabdariffa* L. family "Malvaceae", tribe "Hibiceae". The most important species is *Hibiscus sabdariffa* var. *altissima* Wester, an erect, sparsely-branched annual plant that grows to about 16 ft (4.8 m) high, which is cultivated for its juice - like fiber in India, the East Indies, Nigeria and Tropical America. The stems of this variety are green or red and the leaves are green, sometimes spiny and not used for food. This type at times have been confused with kenaf, *Hibiscus cannabinus* L. a similar but more widely exploited fiber source. The other type of Roselle, *Hibiscus sabdariffa* var. embraces shorter, fleshy with green, red - stem, inedible calyces (Morton, 1987). The last one is commonly known as hibiscus and grows in many tropical and subtropical countries as well as one of highest volume especially botanical products in international commerce. Hibiscus is a fiber, with swollen calyces that are the plant part of commercial interest. As flowers fall off, the bright red calyces swell. These are harvested dried and sold as the herbal tea and beverage industries. The flavour is a combination of sweet and tart, similar to berry. In addition to international markets, there are extensive local and regional markets, where it is processed into hot and cold herbal beverages, jellies, confectionaries and other products. In some African countries, it is called *beri* in the Dagari language, *isapa* in Yoruba, *yakuwa* in Hausa, *mnyanya oji* in Igbo and it is *sour-sour* in Sierra Leone. In the Sudan the plant is called *karkade*, Sahelian countries it is called *dah*, while in Senegal and surrounding countries it is referred to as

bissap. The French name is *oseille de Guinee* or *roselle*. In the Caribbean area the crop is known under the name of 'Jamaican sorrel' or 'Florida cranberry'. It is used in urban areas; it is the calyces of the flower that is the article of trade and the most important part of the plant (Schipper, 2002). The water extract in calyces is rich in carotenoids (especially beta-carotene) and ascorbic acid. The calyces of *Hibiscus sabdarifa* have also been found to be rich in vitamin and other antioxidant and also minerals (Craig, 1999). The physico-chemical 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 a predominant organic acid in Halics. Halics was found to contain higher amount of ascorbic acid compared to orange and mango (Odigie *et al.*, 2003). It was found to be fair source of vitamin A. It is also rich in riboflavin, niacin, calcium and iron . It also contains antioxidants including, flavonoids, gossypetine, hibiscetine and soddertine. Some of the anthocyanins of Roselle indentified by chromatographic process include delphinidin-3-sambubioside, cyaniding-3- sambuboiside and delphinidin -3-glucose (Hung, *et al.*, 2004) They are also known for their unique flavour characteristic that makes them appealing to taste. Roselle drink had been improved nutritionally by producing fruit-flavoured Roselle drink which are richer in vitamin and minerals by addition of different fruits (Odigie *et al.*, 2003).

The most exploited part of a roselle plant is its calyces that are obtained by removing the calyces or petal of the flower from it capsules containing the seeds. Calyces are used for the preparation of herbal drink, cold and warm beverages jams and jellies. The brilliant red colour and unique flavour gives it valuable products (Morton, 1987). Calyces from the roselle flower, which are very high in vitamin C are processed to produce juices. Duke and Atchley (1984) reported that every 100g of fresh calyx contains 2.85 μ g vitamin D, 0.04mg vitamin B1, 0.6mg vitamin B2, and 0.5mg vitamin B complex. Furthermore, other studies have reported that it is good in reducing hypertension (Amin *et al.*, 2008). Ibrahim (2006) reported that drinking 350cm³ per day supplements the maintenance of minimum standard of vitamin C requirement of 60mg/l by human body and that every 10cm³ of sorrel drink contains 1.717mg/l of vitamin C. Roselle

contains three time more vitamin C than Black Currant (*Ribes nigram* L.), nine time more than citrus (*Citrus sinensis* L) fruit. In sudan, the leave are eaten green or dried, cooked with onion and ground nuts. In malaysia, the leaves are also eaten as vegetables after cooking. The calyces are used for tea and as a base for jam (Amin, 2008).

METHODOLOGY

Sample Collection and Preparation

The plant was collected from Ibrahim Badamasi Babangida University, Lapai, farm, Niger State, Nigeria and identified at the Herbarium section of the Department of Biological Science of Ibrahim Badamasi Babangida University, Lapai, as *Hibiscus sabdariffa*. The calyces were air dried for two weeks and ground to uniform powder using wood type of mortal and pestle. Trace metal analysis were carried out on dried sample of the *Hibiscus sabdariffa* calyces. All reagents used were of analytical reagent grade. Distilled water was used in the preparation of all aqueous solutions. All solutions were stored in amber colour bottles.

500mg/L Zinc Standard Solution

The solution was prepared by dissolving 0.50g of pure zinc metal in to 10cm³ Concentrated HCl in 1dm³ volumetric flask and made up to the mark with distilled water (AAS Data Book, 1979).

1000mg/L Copper Standard Solution

Dissolve 3.7980g of (Cu (NO₃)₂.3H₂O in 250ml. of deionised water. Dilute to 1 litre in a volumetric flask with deionised water (AAS Data Book, 1979).

1000mg/L Iron Standard Solution

The solution was prepared by dissolving 1g of iron granules in to 20cm³ of 5M Hydrochloric acid in 1dm³ volumetric flask and made up to the mark with distilled water (AAS Data Book, 1979).

Lead Standard Solution

The solution was prepared by dissolving 1g of lead metal in to 50cm³ of 2M nitric acid in 1dm³ volumetric flask and made up to mark with distilled water (AAS Data Book, 1979).

Atomic Absorption Spectrometer (AAS) measurement.

5.0g of sample in the powdered form was accurately weighed and digested in (5:1) mixture of nitric acid and perchloric acid.

After digestion, few drops of concentrated HCl was added. The solution was heated gently and then filtered. The entire filtrate was diluted up to 100cm³ with distilled water. The filtrate solution was used for analysis of elements of interest by AAS using suitable hollow cathode

lamps. The elements was analysed by AAS techniques by measuring the absorbance of the species at its resonance wavelength. The concentrations of various elements were determined by relative method using A.R. grade reagents.

RESULTS AND DISCUSSION

Table 1: Proximate Analysis of Hibiscus sabdariffa calyces (Light green, Bright red and Dark red).

| | Light green (Mg/L) | Bright red (Mg/L) | Dark red (Mg/L) |
|--------|--------------------|-------------------|-----------------|
| Iron | 22.63 | 25.75 | 24.85 |
| Zinc | 0.220 | 0.234 | 0.240 |
| Copper | 0.645 | 0.325 | 0.447 |
| Lead | ND | ND | ND |

Iron is an essential element for human beings and animals as it is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, proteins and fats to control body weight, which is a very important factor in diabetes mechanism. The results indicates that the concentrations of Fe in the *Hibiscus sabdariffa* calyces (Light green, Bright red and Dark red) samples studied are (22.63, 25.75 and 24.85)Mg/L respectively. The result suggest that high amount of Fe in the samples investigated which may arise from the fact that the soil nature and its topology could influence the chemical content of the plant as the dietary limit of Fe in the food is reported to be in the range 10 – 60mg/day (Kaplan, Pesce and Kazmierczak, 1993; ASTM, 2004; ATSDR, 2005). Low Fe content causes gastrointestinal infection, nose bleeding and myocardial infarction. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells. Iron deficiency is the most prevalent nutritional deficiency in human and is commonly caused by insufficient dietary intake, excessive menstrual flow and anemia (Reddy and Chidambaram, 1987; Sigel, 1978; Hunt, 1994).

Concentration of Zn in the samples was found to be (0.220, 0.234 and 0.240)Mg/L respectively as presented in Table above. Zinc is an essential trace element for plant growth

and also plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception and also causes loss of sense of touch and smell. An excellent treatment for anorexia and bulimia uses high dose of zinc, beyond the recommended daily value of 1.5 to 3.0mg. Zinc deficiency is characterized by recurrent infections, lack of immunity and poor growth, Growth retardation, male hypogonadism, dermatitis, poor wound healing, impaired immune function, skin changes, poor appetite and metal lethargy are some of the manifestations of chronic zinc-deficiency human subject (Prasad, 1982; Sand, 1981; Elinder, 1986; Contran et al., 1989). Zinc is necessary for the growth and multiplication of cells (enzymes responsible for DNA and RNA synthesis), for skin integrity, bone metabolism and functioning of taste and eyesight (Thunus and Lejenne, 1994). Concentration of 5.0mg/l have been reported as minimum acceptable level since above this concentration cause bitter astringent taste and opulence in alkaline water (ASTM, 2004; ASTDR, 2005 and Lewis, 1993).

Copper is an essential enzymatic element needed for normal plant growth and development but can be toxic at excessive level. Toxicity can occur if its concentration in plants is higher than, 20 – 100mg/L (DW) (dry weight). The concentration of copper in the samples studied; *Hibiscus sabdariffa* calyces (Light green, Bright red and Dark red) were

(0.645, 0.325 and 0.447Mg/L) as presented in table above. High level Cu may cause metal fume fever with flu like symptoms, hair and skin decoloration, dermatitis, irritation of the upper respiratory track, metallic taste in the mouth and nausea. WHO has recommended the lower limit of the acceptable range of copper as 20ug/mg body weight per day. Copper deficiency results in anemia and congenital inability to excrete copper (ATSDR, 2005; ASTM, 2004; IPCS, 1998; WHO, 1998).

Concentration of Pb in *Hibiscus sabdariffa* calyces (Light green, Bright red and Dark red) was not detected (Nd) in the samples studied as represented in table above. Lead is a non-essential element that can be introduced into the human organism by inhalation, ingestion and absorption. It is an undesirable trace metal abundantly found in soil, vegetations, animals and foods. Although its concentration can be relatively high in the liver and kidney, lead is mainly deposited in the bones. The deleterious properties of lead are particularly significant in the central nervous system, kidneys and livers. (Kleassen, 2001; ATSDR, 2005; ASTM, 2004). Studies have shown that prescribed limit for Pb contents in herbal medicine is 5-10 mg/l while the dietary intake limit for Pb is 3mg/week. It has serious cumulative body poison as lead inhibits several key enzymes involved in the overall process of haemo-synthesis thereby damaging the organs involve as it accumulates (Wedepohl, 1978; WHO, 2000).

REFERENCES

- Ahmed, S. (2007). Introduction of plant constituents and their tests, lecturer in Department of Pharmacology and phytochemistry, Faculty of Pharmacy, Hamdara Nagar, New Delhi. 2 -3.
- Amin I. and Emmy, H. K. I., (2008). Roselle Seed Nutritional Composition, Protein Quality and Health Benefit p 2-3
- AOAC (1990). Official Methods of Analysis. Association of Official Analytical Chemistry, Washington D.C. 15th Ed.
- ASTM (2004). American Society for Testing and Materials. International West Conshohocken PA 19428—2959, Section 11 water and environmental Technology, vol II 01.
- ATSDR (2005). Agency for Toxic Substance on Disease Registry. Toxicological profile for zinc. US Department of Health and Human Services, Atlanta, Georgia.
- Contran, R. S., Kumar, V. and Robbins, S.L. (1989). Robbins pathologic basis of disease. 4th ed. Philadelphia, PA: W.B. Saunders company, 461.
- Craig, W. J. (1999). Health- promoting properties of common herbs. Am J Clin Nutr. 70 (suppl): 491S-499S.
- Duke J. A. and Atchley A. A. (1984). Proximate analysis in christie B. R (ed). The hand book of plant science in agriculture, CRC press, Inc., Boca Raton.
- Elinder, C.G. (1986). Zinc. In Friberg, L; Nordberg, F.F; Vouk, V eds. Handbook on toxicology of metals. Vol II, New York, NY: Elsevier science publishers B.V. 664 – 679.
- Guyton, K.Z. and Kensler, T.W. (1993). Oxidation mechanisms in carcinogenesis, Br med Bull. 49: 523.
- Hung, H.C. Joshipura, K.J. Jiang, Hu, F.B., Hunter, D., Smith – Warner, S.A., Coldutz, G.A., Rosner, B. Spiegelman, D., and Willet, W.C. (2004). Fruit and vegetable intake and risk of major chronic disease J. Natl Cancer Inst. 96: 1577.
- Hunt, J. R. (1994). Bioavailability of fe, zn and other trace minerals for vegetarian diets. Am. J. Clin. Nutr. 78: 633 - 639.
- IPCS (1998). Environmental Health Criteria 200, Copper. International Programme on Chemical Safety, United Nations Environmental Programme (UNEP), International Labour Organization (ILO), and World Health Organization,(WHO) Geneva.
- Jansen, M.C. Mc Kenna, D., Bueno-de-mesquita, H.B., Feskens, E.J. Streppel, M.T., Kok, F.J., and Kromtrout, D. (2004). Reports: quantity and variety of fruit and vegetable consumption and cancer risk. Nutr, Cancer 48: 142.

- Kaplan, L.A., Pesce, A. J. and Kazmierczak, S.C. (1993). Theory, Analysis, Correlation, In: Clinical Chemistry 4th Ed. P 707.
- Lewis, R. J. (1993). Hawleys Condensed Chemical Dictionary 12 ed. Van Nostrand Reinhold, New York, USA. P254.
- Morton, J.F. (1987). Roselle. In: Fruits of warm climate, (CF Dowling ed). Media, Inc. Greensboro, NCP. 281-286.
- Odigie, I. P., Ettarh, R. R., Adigun, S. A. (2003). Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1 C hypertensive rats. *J Ethnopharmacol* 86: 181-185.
- Prasad, A. S. (1982). Clinical, Biochemical and Nutritional Aspects of Trace Elements. Alane R. USS Inc., New York.
- Reddy, M. B.; Chidambaram, M. V.; Bates, G.W., (1986). Iron Transport in Microbes, Plants and Animals. VCH, New York.
- Sandstead, H.H. (1981). Zinc in human nutrition. In: Bronner, F; Coburn, J.W. eds. Disorders of mineral metabolism. New York, NY: Academic press 94-159.
- Schipper, R. R. (2002). African Indigenous Vegetables. An overview of the cultivated species. 133.
- Sigel, H. (1978). metal in Biological Systems. Marcel Dekker New York.
- Singh, G., Sharma, P.K. Dudhe R. And Singh, S. (2010). Biological activities of *Withania somnifera*, *Annals of Biological Research*. 1(3): 56-63
- Wallstrom, P., Wirfalt, E., Janzon, L., Mattisson, I., Elmstahl, S., Johansson, U., and Berglund, G. (2000). Fruit and vegetable consumption in relation to risk factors for cancer: a report from the Malmo Diet and Cancer Study. *Public Health Nutr*.3: 263.
- Wedepohl, K.H. (1978). Hand book of Geochemistry Ex-Editor, Springer Verlag Berlin Heidelberg; New York, 11 – 15.
- Weinberger, J.H. (1991). Nutritional approach to cancer prevention with emphasis on vitamin, antioxidants and carotenoids. *AM J Clin. Nutr.* 53: 2265.
- (WHO) World Health Organization (1998). Quality Control Methods for Medicinal Plant materials, WHO Geneva Switzerland.