

Determination of Heavy Metals and Phytochemical analysis of some selected Vegetables grown at Kalambaina Area Sokoto.

ABSTRACT

Heavy metals and phytochemicals were investigated in some selected vegetables grown at Kalambaina area of Sokoto. The levels of heavy metals and phytochemicals were analyzed using Atomic Absorption Spectrometric (AAS) and standard analytical procedures respectively. The concentration of heavy metals were found to be higher in *Amarantus specie*. Pb; (1.3292 ± 0.014 mg/kg), Ni; (0.2785 ± 0.011 mg/kg), Cr; (0.1136 ± 0.012 mg/kg), Cd; (0.0032 ± 0.011 mg/kg) and *spinacea oleracea* Pb; (0.2183 ± 0.011 mg/kg), Ni; (0.0165 ± 0.020 mg/kg), Cr; (0.0986 ± 0.011 mg/kg) Cd; (0.0062 ± 0.014 mg/kg), however, low concentrations were observed in *Allium cepa* Pb; (0.0764 ± 0.011 mg/kg), Ni; (0.0032 ± 0.011 mg/kg), Cr; (0.1333 ± 0.015 mg/kg), Cd; (0.0046 ± 0.020 mg/kg). The results of the study for the three (3) vegetables revealed the concentrations of the heavy metals are within the World Health Organization (WHO) and Standard Organization of Nigeria (SON) safe limits guidelines with the exception of Cr and Pb. Phytochemical screening of methanolic extracts of the vegetables showed the presence of alkaloids, saponins, tannins, flavonoids, phenolics and terpenoids. Therefore, this study revealed that the vegetables can be use as efficient source of secondary metabolites.

Keywords; Heavy metals, Phytochemicals, Kalambaina and Atomic Absorption Spectroscopy

1.0 INTRODUCTION

Plants are nature's gift to mankind in terms of providing us with food, oxygen, as well as shelter. Since time immemorial, they have served as the first line of defence used by our forefathers to fight diseases such as Diarrhea, Cholera and Malaria [1]. Plants are conveniently separated into those which are edible, those which serve as a source of drugs or spices, and those that are of ornamental value etc. Although almost intensively cultured plant rightly comes under the domain of horticulture, primary effort is centered on the various traditional "garden" plants. The Horticulturist divides the edible garden plant into vegetables and fruits [2].

Vegetables are important edible crops and are an essential part of the human diet. They are rich in nutrients required for human health, and are an important source of carbohydrates, vitamins, minerals, and fibers [3]. Heavy metals can be readily taken up by vegetable roots, and can be accumulated at high levels in the edible parts of vegetables, even heavy metal in soil at low levels. In many countries and regions, vegetables are exposed to heavy metals by various means, thus vegetable consumption can cause adverse health effects [4].

Heavy metals are natural constituents in nature, usually occurring in low concentration under normal conditions. Heavy metals contamination of vegetables cannot be underestimated as these food stuffs are important components of human diet [5]. Despite of the bright prospects and enormous potential need of vegetables and fleshy fruits production in Nigeria, there are challenges such as Poor growth and harvesting techniques, lack of storage facilities, inadequate processing facilities, and improper packaging and pests' protection management procedures. These factors mentioned may lead to the contamination of the vegetables and fruits with the heavy metals. Anthropogenic activities can cause elevated levels of these metals in various parts of the ecosystem and thus environmental pollution by heavy metals may occur via various diffused and point sources [2]. Assessment of heavy metal compositions of vegetable is one of the most important method used for monitoring environmental pollution, as the deficiency or excess of the elements is known to cause a number of serious metabolic growth, physiological and as well as toxic effect [6]. The present study is aim at investigating the levels of some heavy metals (Cd, Cr, Ni and Pb) and phytochemicals in edible portion of *spinacea oleracea*, *Allium cepa* and *Amaranthus specie* grown at kalambaina area Sokoto.

2.0 Materials and methods

2.1 Chemicals and Reagents

The reagents used for the study included hydrochloric acid (HCl), trioxonitrate acid (HNO₃), sodium hydroxide (NaOH), ferric chloride, Wagners reagent (iodine in potassium iodide), sulphuric acid (H₂SO₄), sodium chloride (NaCl), perchloric acid (HClO₄), chloroform, methanol, and distilled water. All other chemicals used were of analytical grade and purchased from standard manufactures.

2.2 Samples collection

Fresh samples were collected in a pre-cleaned plastic bags from Kalambaina area Sokoto down Cement Company of Northern Nigeria. The samples were identified and authenticated at the Department of Biological science Sokoto State University Sokoto. The collected samples were washed separately under running tap water before cut down into smaller pieces using knife and dried at room temperature for about 2 weeks to a constant weight. The samples were grounded into a fine powder, sieved and stored in plastic jar labeled for analysis.

2.3 Extraction of plant samples

The procedure that was used for the extraction of the active ingredients from the plant parts in this research was extraction by evaporation using 70% methanol. This was done by suspending 50g of the samples in 500ml of 70% methanol. The extraction was allowed for 24 hours at room temperature on shaker. Each sample was separated using Whatman filter paper, the filtered liquid was then dried in evaporator at 35°C for about 4 hours to get extract.

2.4 Digestion procedure

A 2.0 g of the samples were weighed into Kjeldahls flask mixed with 20 cm³ of concentrated sulphuric acid, concentrated perchloric acid and concentrated nitric acid in the ratio 1:4:40 by volume respectively and left to stand overnight. Thereafter, the flask was heated at 70°C for about 40 min and then, the heat was increase to 120°C. The mixture turned to black after some time [7]. The digestion was completed after the solution became clear and white fumes appeared. The digest was diluted with 20 cm³ of distilled water and boiled for 15 min. Solution was then allowed to cool, it was transferred into 100 cm³ volumetric flasks and diluted to the mark with distilled water. The sample solution was then filtered through a filter paper into a screw capped polyethylene bottle, the procedure was repeated for all the samples.

2.5 Determination of heavy metals

The method applied for the assessments of heavy metal concentration of each sample of plant materials, after the digestion of the samples was by using the Atomic Absorption Spectrometric techniques as described previously [8].

2.6 Phytochemicals screening

2.6.1 Alkaloids

Wagner's test: 1cm³ of each extracts were treated with 2 drops of Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids in the extracts [9]

2.6.2 Flavanoids

Alkaline reagent test: 3cm³ of each extract were treated with 1cm³ of 10% NaOH solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids in the extracts.

2.6.3 Saponins

Foam method; 0.5g of each extract were treated with 5ml of distilled water, and mixture was shaken vigorously, the production of foam which persisted in few minutes indicated the present of saponins in the extracts.

2.6.4 Phenolics

Ferric chloride test; 2.0ml of each extract were treated with 3-4 drops of 10% ferric chloride, formation of bluish black color indicates the presence of phenolics compound in the extract.

2.6.5 Tannins

Ferric chloride test; 2 drops of 5% FeCl₃ was added to 1 cm³ of each extract. A greenish precipitate indicated the presence of tannins in the extracts.

2.6.6 Terpenoid

Salkowski method; 1ml of each extract was treated with 2ml of chloroform and few drops of sulphuric acid, it was shaken and allowed to stand for few minutes, appearance of reddish brown color at the lower layer indicates the presence of terpenoids in the extracts [10]

110 **3.0 RESULTS AND DISCUSSION**111 **3.1 RESULTS**

113	Metals	<i>Spinacea oleracea</i>	<i>Allium cepa.</i>	<i>Amarantus Specie</i>	WHO STD.	NIG STD.
114	(Cd) mg/kg	0.0062 ± 0.014	0.0046 ± 0.020	0.0032 ± 0.011	0.05	0.003
115						
116	(Cr) mg/kg	0.0986 ± 0.011	0.1333 ± 0.015	0.1136 ± 0.012	0.05	0.05
117						
118	(Ni) mg/kg	0.0165 ± 0.020	0.0032 ± 0.011	0.2785 ± 0.011	0.05	0.02
119						
120	(Pb) mg/kg	0.2183 ± 0.011	0.0764 ± 0.011	1.3292 ± 0.014	0.03	0.01
121						

124 NIG STD. = Nigerian Standard based on SON (2011)

126	Phytochemical	<i>Spinacea oleracea</i>	<i>Allium cepa</i>	<i>Amarantus specie</i>
127	Alkaloid	-	-	+
128	Flavanoid	+	++	-
129	Saponin	-	-	++
130	Tannins	++	++	++
131	Phenolics	+	++	-
132	Terpenoids	++	+++	-
133				

136

137

3.2 DISCUSSION

The concentration of heavy metals (Cd, Cr, Ni and Pb) were investigated in *Amarantus specei*, *Allium cepa* and *Spinacea oleracea* grown at Kalambaina Area, Sokoto. The results obtained in (Table 1) showed that there is higher concentration of Lead (Pb) in *Amarantus specei*, ($1.3292 \pm 0.014\text{mg/kg}$), *spinacea oleracea* ($0.2183 \pm 0.011\text{mg/kg}$) and *Allium cepa* ($0.0764 \pm 0.011\text{mg/kg}$), the concentration of lead is above the maximum allowable concentration of World Health Organization (WHO) and Standard Organization of Nigeria (SON). Our result supports the study conducted by Okerulu et al. which also showed that there is high concentration of lead (Pb) in plants spp [11].

Our study also showed that there is higher concentration of Chromium in *Allium cepa* ($0.1333 \pm 0.015\text{mg/kg}$), *Amarantus specei* ($0.1136 \pm 0.012\text{mg/kg}$) and *Spinacea oleracea* ($0.0986 \pm 0.011\text{mg/kg}$) (Table 1) when compared to the maximum allowable concentrations of both WHO and SON (MAC, 0.05mg/kg and 0.05mg/kg respectively), This is similar to the study which investigated Chromium toxicity in some vegetables which also high Chromium concentration [12].

The concentration of Nickel was low in *Allium cepa* ($0.0032 \pm 0.011\text{mg/kg}$) and *Spinacea oleracea* ($0.0165 \pm 0.020\text{mg/kg}$), but in high in *Amarantus specei* ($0.2785 \pm 0.0011\text{mg/kg}$) according to WHO and SON standard.

Cadmium (Cd) concentrations obtained were ($0.0062 \pm 0.014\text{mg/kg}$), in *Spinacea oleracea* ($0.0046 \pm 0.020\text{mg/kg}$) in *Allium cepa* and ($0.0032 \pm 0.011\text{mg/kg}$) in *Amarantus specei* (Table 1) which revealed low MAC for WHO (MAC, 0.05mg/kg), but high SON (MAC, 0.003mg/kg). This study contradicts research conducted by Satarug et al which showed that Cadmium is predominantly found in plants spp [13].

Phytochemicals are biologically active occurring chemicals compound found in plants which provide health benefits for human further than those attributed to macronutrients and micronutrients [11]. The results of qualitative phytochemical screening of this research revealed the presence of biologically active compounds such as Tannins, Alkaloids, Saponins, Flavoniods and Phenolic at different levels.

The phytochemical screening showed alkaloids were present in *Amarantus specei* leafs (+) but absent in *Spinacea oleracea* and *Allium cepa* (-) (Table 2). This contradicted the study which reported that alkaloids

were present in all vegetables [14]. The presence of alkaloids in *Amarantus specei* indicated that the leaf can be used for the treatment of malaria, cold, cough, diabetes, and hypertension [15].

Flavonoids were found to be moderately present in *Allium cepa* (++) , slightly present in Spinach (+) and absent in *Amarantus specie* (-). Flavonoids plays an important role in protecting biological system on macromolecules such as lipids, carbohydrates and proteins [16]. Extracts of Flavonoids are known to have anti-inflammatory, anti-allergic, anti-viral anti-spasmodic and diuretic effect [17].

Saponins were found to be moderately present in *Amarantus specie* (++) but absent in *Spinacea oleracea* and *Allium cepa* (-) respectively. This agrees with the finding that reported the present of saponins in *Amarantus specie* [14]. Saponins are known to be immune boosters. Extracts of plants rich in saponins are said to demonstrate anti-inflammatory, hemolytic, allelopathic, cholesterol lowering and anti-cancer properties [17].

Tannins were found to be moderately present in *Allium cepa*, *Spinacea oleracea* and *Amarantus specie* (++) . Tannins are chelating agents for metals and can form complexes with macromolecules through the process, essential substrates and enzymes of microorganisms are depleted leading to cell death [11]. Tannins are good anti-microbial agent with precipitate protein thereby providing water proof layer on the skin when used externally or protect the underlying layers of the skin and limit the loss of fluid. They are also known to be good anti-viral agents [11].

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. The phytochemical screening revealed the present of phenolic compound in *Allium cepa* and *Spinacea oleracea* (+) but absent in *Amarantus specie*. Extract of plants rich with phenolic compounds plays an important role in biological activities such as antiulcer, antidepressant, antioxidant, cytotoxic and antitumor [18].

4.0 CONCLUSION

The presence study confirmed the presence of heavy metals (Cd, Cr, Ni and Pb) in vegetables (*Spinacea oleacea*, *Allium cepa* and *Amarantus specie*) grown at Kalambaina area Sokoto. However, the level of heavy metals were currently within the WHO and SON safe limits guidelines with the exception of Cr and Pb which exceeded the safe limit guidelines in all the vegetables. The study has also confirmed the presence Alkaloids, flavonoids, tannins, phenolic and saponins in all the vegetables. The presence of many phytochemicals in the vegetables helps to enrich the immune system and other haematological parameters in human body.

5.0 RECOMMENDATIONS

Further studies should be carried out to determine the concentration of heavy metals in soil and water of the study area. The study should also be extended to different areas of Kalambaina.

COMPETING INTERESTS

The authors have not declared any competing interests exist.

REFERENCES

1. Mathias T. A, Christopher L, Regina A, Isaac T, Kofi B, Abigail A. Phytochemical, Antioxidant and Cytotoxicity of Hdoroethanolic extracts of *crotalaria retusa* L. World Journal of Pharmaceutical Research.2016;5 (2): 162-179.
2. Gwana A.M, Bassey E, Hauwa LB, Halima B, Mustapha UM, Ali M, Laku D. Appraisal of Some Heavy Metals and Elements in Irrigated Vegetables and Fruit Cultivated in College Farm, Maiduguri, Nigeria. International Journal of Applied Agricultural Sciences.2017; 3(3): 78-86.
3. Hu J, Wu F, Wu S, Cao Z, Lin X., Wong M.H. Bioaccessibility, dietary exposure and human risk assessment of heavy metals from market vegetables in Hong Kong revealed with an in vitro gastrointestinal model. Journal of Chemosphere.2013; 9(1):455–461.
4. Yang Y, Zhang F.S, Li H.F, Jiang, R.F. Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. J. Environmental management and public health. 2012; 90: 1117–1122.

- 217 5. Abdulmojeed O. L, Abdulrahman A. A. Analysis of heavy metals found in vegetables from some
218 cultivated irrigated gardens in the Kano metropolis, Nigeria. Journal of Environmental Chemistry
219 and Ecotoxicology. 2011;3(6):142-148.
- 220 6. Agrawal. Effect of heavy metals contaminated vegetables, Indian journal of pharmacology.2011;
221 5: 4-6.
- 222 7. Erwin JM, Ivo N. Determination of Lead in tissues: A pitfall due towet digestion procedures in the
223 presence of sulphuric acid. 1992;1(7): 23-26.
- 224 8. A.O.A.C. Association of official analytical chemistry. 1990 18th edition, Washington DC. U.S.A.
- 225 9. Wagners. Method for detection of alkaloid in biological using wagners reagent (iodine in
226 potassium iodide). Internationalepharmaceuticasciencia.1991;1 (1).
- 227 10. Salkowski. Method for determination of terpenoids. Internationalepharmaceuticasciencia. 1951;1
228 (1)
- 229 11. Okerulu I. O, Onyema C.T, Onwukeme V.I., Ezech C.M. Assessment of Phytochemicals,
230 Proximate and Elemental Composition of *Pterocarpussoyauxii* (Oha) Leaves. American Journal of
231 Analytical Chemistry.2017; 80:406-415
- 232 12. Ghani A. Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of
233 Brassica juncea L. gyptianAcadademic Journal of Biological Science. 2011;2(1):9–15.
- 234 13. Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure, and health
235 outcomes. Ciência&SaúdeColetiva. 2011;16(5):2587–2602
- 236 14. Ajiboye A.A, Fadimu O. Y, Ajiboye M.D, Agboola D. A, Adelaja A. B,Bem A.A. Phytochemical and
237 Nutritional Constituents of Some Common Vegetables in South-West, Nigeria. Global Journal of
238 Science Frontier Research. 2014;14(1).
- 239 15. Dike M.C. Proximate and Nutrient Composition of Some Fruits, Seeds and Leaves of Some Plant
240 Species at Umudike, Nigeria. ARPN-Journal of Agricultural and Biological Sciences.2010: 5: 7-
241 16.
- 242 16. Atmani D, Nassima C, Dina A, Meriem B, Nadjat D, Hania B. Flavonoids in Human Health: From
243 Structure to Biological Activity.Current Nutrition & Food Science.2009; 5: 225-237.

- 244 17. Mercy Tope Ajibua. Qualitative and Quantitative analysis of the Phytochemicals in some selected
245 species of the genus *phyllanthus* in Zaria, Nigeria. 2015
- 246 18. Ghasemzadeh, A, Jaafar HZE, Rahmat A. Antioxidant activities, total Phenolics and flavonoids
247 content in two varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). *Molecules*. 2010;
248 15: 4324-4333.
249