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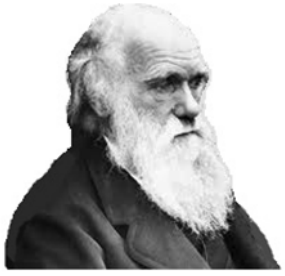

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Determination of Total Phenolic Content and Some Selected Metals in Extracts of *Moringa oleifera, Cassia tora, Ocimum gratissimum, Vernonia baldwinii* and *Telfairia occidentalis* Plant Leaves

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ABSTRACT

The main objective of this research is to determine the content of metals (Ca, Cu, Fe, Mg and Zn) and total phenols in different plant extracts of *Moringa oleifera, Cassia tora, Ocimum gratissimum, Vernonia baldwinii* and *Telfairia occidentalis*. Contents were determined using Atomic Absorption Spectroscopy. The results indicate that *Moringa oleifera* plant extracts range from 0.25 ±0.00 to 6.13 ±0.30 mg/kg, *Cassia tora* plant extracts - 0.17 ±0.03 to 7.48 ±0.06 mg/kg, *Ocimum gratissimum* plant extracts - 0.18 ±0.00 to 5.43 ±0.12 mg/kg, *Vernonia baldwinii* and *Telfairia occidentalis* plant extracts - 0.21 ±0.03 to 7.86 ±0.12 mg/kg and 0.17 ±0.00 to 4.52 ±0.06 mg/kg, respectively. The results also revealed a lower abundance of heavy metals. The total phenolic content was determined using the modified Folin-Ciocalteu method. Herein, the phenolic content in *Moringa oleifera* was 8.50 ±1.23 mg, Garlic Acid Equivalent g^{-1} (mg GAE g^{-1}), *Cassia tora* - 30.00 ±0.00 mg GAE g^{-1}, *Ocimum gratissimum* - 45.00 ±1.41 mg GAE g^{-1}, *Vernonia baldwinii* - 49.00 ±1.14 mg GAE g^{-1}, and *Telfairia occidentalis* - 46.67 ±0.27 mg GAE g^{-1}. We found the lowest total phenol content in *Moringa oleifera.* This also possessed a high chelating activity. In contrast, *Vernonia baldwinii* contained the highest total phenol content, but had low chelating activity. The plant extracts with high levels of phenolic compounds exhibited good antioxidant activity.

***Keywords*:** Phenol, metals, plants, diets, Nigeria herbs, antioxidant

1. INTRODUCTION

Phenols are sometimes called phenolics and are class of compounds consisting of a hydroxyl group - (OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is the phenol which is also called carbolic acid C_6H_5OH. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecules (Khoddami *et al*., 2013). Phenols have higher acidities due to the aromatic rings coupling with the oxygen and a relative loose bond between oxygen and hydrogen. The acidity of the hydroxyl group in phenols is commonly intermediate between that of aliphatic alcohols and carboxylic acids. The basic structure of phenols includes an aromatic ring and a hydroxyl group. Depending on the number of phenolic units, the location and the number of hydroxyl group, the phenolic family includes over 8000 compounds (Gulcin *et al*., 2013). Phenols, depending on their derivatives, are divided into three main groups: (a) phenolic acids that occur as hydroxylated benzoic acid derivatives, (b) phenolic acids as cinnamic acid derivatives, and (c) glycosidic phenylpropanoids (Skerget *et al*., 2005). There is an increasing interest in the biological effects of phenols, since there are to be found numerous studies connecting phenolic content and antioxidant, antimicrobial, and anticancer activities of fruits, vegetables, flowers, leaves, and seeds (Bursal *et al*., 2013). Antioxidants are chemical compounds that can quench reactive radical intermediates formed during oxidative reactions. The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines (Ozta *et al*., 2015). The majority of natural antioxidants are phenolic compounds (Gocer *et al*., 2011).

Vegetable species differ widely in their ability to take up and accumulate heavy metals, even among cultivars and varieties within the same species (Zhu *et al*., 2007). Louis *et al*., (2017) reported that coconut fluid and milk obtained from Takum, (Taraba State) Owerri, (Imo State) and Jimeta, (Adamawa State) were analyzed for the presence of the metals Fe, Pb, Cu, and Zn. The results obtained were with the following trends; in coconut fluid, Fe>Zn>Cu>Pb, while in the coconut milk it follows this trend, Fe>>Zn>Cu>Pb. Alexander *et al*. (2006) reported that Pb was significantly accumulated in lettuce and onion, while Cd was accumulated to the greatest extents in spinach and lettuce. Yang *et al*. (2009) found that Chinese leek, pak choi, and carrot had higher Cd concentrations in their edible parts than radish, cucumber, and tomato. Säumel *et al*. (2012) reported that Zn concentrations in green beans, tomato, potato, kahlrabi, and carrots were significantly lower than the concentrations in leafy vegetables. Cd accumulation in vegetable species decreased in the order of leafy vegetables > solanaceous vegetables > root vegetables > allimus vegetables > melon vegetables > legumes vegetables (Yang *et al*., 2010).

Moringa oleifera is an edible plant. A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (Kumar *et al*., 2010). Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants like β-carotene, vitamin C, and flavonoids (Gowrishankar *et al*., 2010). *Cassia tora* is a wild crop plant that belongs to the *Caesalpinaceae* plant family and genus *Cassia*. *Cassia tora* seeds are hard to beat in their quality and affordability. They are bold and small seeds. These seeds are also shiny and are duly machine cleaned. These *Cassia tora* seeds are available into many ranges such as *Cassia tora* seed and natural *Cassia tora* seeds. *Ocimum gratissimum* L. is a shrub belonging to the family *Lamiaceae*. It is commonly known as Scent leaf or Clove basil and is found in many tropical countries. Africa and Asia are, however, the two continents where

most variants of the plant exists (Mann, 2012). *O. gratissimum* is found in the tropical and warm temperature regions, such as India and Nigeria (Nwinyi *et al*., 2009).

Telfairia occidentalis is a tropical Vine grown in West Africa as a leafy vegetable and for its edible seeds. Common names for the plant include fluted gourd and fluted Pumpkin. It is known as Ugu (Igbo language) in eastern parts of Nigeria. *Telfairia occidentalis* leaves are sometimes called Pumpkin leaves in English. The plant is dioecious, perennial, and drought-tolerant. It is usually grown trellised (*Telfairia occidentalis*, 2009). Therefore, the main purposes of this study are Determination of Total Phenolic Content and Some Selected Metals in Extracts of *Moringa oleifera, Cassia tora, Ocimum gratissimum, Vernonia baldwinii,* and *Telfairia occidentalis* Plant Leaves found in Jalingo, Taraba State, Nigeria

2. EXPERIMENTAL

Sample Collection

Fresh parts of five herbal plants leaves *Vernonia baldwinii, Moringa oleifera,Telfairia occidentalis, Ocimum gratissimum* and *Cassia tora* were collected from different areas in Jalingo and Ardo-kola Local Government Area of Taraba State in the North Eastern region of Nigeria. The plant materials were authenticated at the Department of Chemistry Modibbo Adama University of Technology, Yola Adamawa State.

Preparation of Plant Materials

The fresh plant materials were collected and the voucher specimens were numbered 1-5 and kept in Chemistry Research Laboratory of Modibbo Adama University of Technology, Yola Adamawa State. The parts of the plant materials collected were freed from twigs and extraneous matter. Soil grit, sand, and dirt were removed by sifting. In other to remove the remnants of adhering foreign matter, the samples were rapidly and thoroughly washed under tap water and rinsed with distilled water and then shade dried at room temperature for 15 days. After drying, the plant materials were ground to fine powder and transferred into airtight containers with proper labeling for future use.

Determination of selected metals

About 2 g of sample was weighed and transferred into a Silica Crucible and kept in a muffle furnace for ashing at 450 °C for 3 hours and then 5 cm^3 of 6 M HCl was added to the Crucible. Proper care was taken to ensure that all the ash comes into contact with the acid. Further, the acid solution in the Crucible was transferred into Kjehdahl flask and the flask was placed on a heating mantle and digested in order to obtain a clear solution. The final residue was dissolved in 0.1 M HNO_3 solution and made up to 50 cm^3. Working standard solutions were prepared by diluting the stock solution with 0.1 M nitric acid in order to check the linearity.

2. 1. Extraction of plant Materials

Solvent extraction

Crude plant extract extractions were described previously in detail using soxhlet method, as reported by Louis *et al*., (2015). About 10 g of powdered plant material was uniformly packed into thimble and extracted with 125 cm^3 of methanol. The process of extraction continued for

24 hours or till the solvent in Siphon tube of an extractor became colourless. After that, the extract was taken into a beaker and kept on hot plate and heated at 30-40 °C till all the solvent got evaporated. Dried extract was kept in the refrigerator at 4 °C use for future in phytochemical analysis.

Equipments / Apparatus

Atomic Absorption Spectrometer (AAS), UV/Visible Spectrometer, laboratory glass ware, analytical balance, Pestle and Mortar, Sieve, and Whatman filter paper were used for the studies.

Determination of total phenol contents

Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method used by Singleton and Rossi, (1965). An aliquot of the extracts (1 ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of the sodium carbonate solution (20%). Tubes were vortexed for 15 s and allowed to stay at 40 °C for 30 min in order to develop the colour. Absorbance was then measured at 765 nm, using UV-VIS spectrophotometer. The total phenol content was expressed as mg of garlic acid equivalent (GAE) g^{-1}. The result of each assay was obtained from three parallel determinations.

3. RESULTS AND DISCUSSION

Metals content from the dried plant extracts

The results of metals content in all the plant extracts presented in **Table 1** showed that Ca and Mg were the highest and the presence of Cu, Fe and Zn was lowed. The result showed that Ca content was the highest in *Vernonia (*7.86 mg/kg) and the lowest in *Telfairia occidentalis* (4.52 mg/kg). The presence of Mg was higher in *Vernonia* (4.00 mg/kg) and lower in *Telfairia occidentalis* (2.21 mg/kg). Cu had the lowest content in *Cassia tora* (0.17 mg/kg) and *Telfairia occidentalis* (0.17 mg/kg) and its highest content (0.18 mg/kg) was found in *Moringa oleifer*a. Fe was found the highest in *Ocimum gratissimum* (1.58 mg/kg) and the lowest in *Cassia tora* (0.37 mg/kg). Zn was found higher in *Vernonia* (1.53 mg/kg) and also the lowest in *Telfairia occidentalis* (0.87 mg/kg).

The comparison of the results, regarding heavy metals content in the plant from the useful plants at mangrove swamp forest of Southeast Serbia (Louis *et al.,* 2017) with the results of the other authors, showed a considerable *agreement*. The content of copper in Palestinian plants varied from 7.06 to 19.19 mg/kg, and of zinc from 17.38 to 65.85 mg/kg (Khan *et al*., 2008). The copper content in the black tea originating from the region of south India varied between 15.9 and 32.2 mg/kg (Sharma *et al.,* 2009). In the plant from the region of the Southeast Serbia, the highest content was that of Iran, while the contents of Zn, Cu and Mn were significantly lower. In the black tea samples originating from the region of Iran, the copper concentration was within the range from 17.59 to 32.80 mg/kg, and in the water extracts from 1.15 to 1.65 mg/kg (Karimi *et al.,* 2008). The iron concentration in the medicinal plant from Turkey ranged from 2.45 107.4 mg/kg, zinc from 3.90 to 18.00 mg/kg, and copper from 2.45 to 8.10 mg/kg (Basgel and Erdemoglu, 2006).

Comparing our results with the results of the authors from other countries it can be observed that the heavy metals content is smaller. Based on these results, we recommend the

use of the plant extracts with lower abundance of heavy metals. Minerals are inorganic substances present in all body tissues and fluids, and their presence is necessary for the maintenance of certain physicochemical processes which are essential for life (Jabeen *et al.*, 2010).

Table 1. The comparative study of metals content in the plant extracts.

Sample	Ca (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Zn (mg/kg)
Moringa olifera	6.13±0.38	0.25±0.00	1.14±0.05	3.18±0.07	0.62±0.44
Cassia tora	7.48±0.06	0.17±0.03	0.37±0.05	3.64±0.00	1.36±0.03
Ocimum gratissimum	5.43±0.12	0.18±0.00	1.58±0.07	2.79±0.05	1.13±0.00
Vernonia	7.86±0.12	0.21±0.03	1.17±0.07	4.00±0.00	1.53±0.06
Telfairia occidentalis	4.52±0.06	0.17±0.00	0.86±0.20	2.21±0.05	0.87±0.00

*Concentration of metals is expressed as mg/kg

Phenolic content for the plant extracts

The content of the total phenol in the extracts of the investigated plant leaves were determined using *Folin-Ciocalteu* method and were expressed as mg Garlic Acid Equivalent GAE g^{-1} of the dry samples shown in **Table 2**. The total phenolic content of the investigated plant extracts ranged from 8.50 mg GAE g^{-1} to 49.00 mg GAE g^{-1} of the samples. The content of the phenols was the highest in *Vernonia* plant extract and the lowest in *Moringa oleifera* plant extract. This shows that *Moringa oleifera* with low phenol content had good chelating activity but *Vernonia baldwinii* with high phenol content had a weak chelating activity. The high phenolic compounds exhibited good antioxidant activity in the plant extracts (Table 2).

Table 2. The comparative study of total phenolic and total flavonoid content in the plant leaves extracts.

Sample	Total phenolic content[a]
Moringa oleifera	8.50±1.23
Cassia tora	30.00±0.00
Ocimum gratissimum	45.00±1.41
Vernonia	49.00±1.14
Telfairia occidentalis	46.67± 0.27

[a]Expressed as mg of garlic acid equivalent g^{-1} of dry sample,

The content of the phenols represent a pharmaceutical characteristic of the plants. It is known that the content of the polyphenol compounds depends on the genotype, soil conditions

and the difference in the plant ripening. Also the environmental conditions, such as altitude, light, temperature, and the content of the persistent organic pollutants in soil have an effect on the phenylpropanoid metabolism (Louis *et al*., 2015).

4. CONCLUSION

The extracts obtained from the leaves of *Moringa oleifera, Cassia tora, Ocimum gratissimum, Vernonia baldwinii* and *Telfairia occidentalis* contained a high quantity of polyphenolic compounds with the exception of *Moringa oleifera* which contained low phenol content. The high phenolic compounds exhibited good antioxidant activity in the plant extracts. The contents of heavy metals (Zn, Fe, and Cu) were low in the plant extracts. These investigations are obligatory and they are recommended by the European standards in order to prevent poisoning by heavy metals. The investigated plants' leaves from the Northeast region of Nigeria could be suitable for the preparation of herb extracts due to the low contents of heavy metals, the high content of phenolic compounds and a high antioxidant activity. Moreover, these plant extracts should be investigated *in vivo* to better understand their safety, efficacy, and properties. Overall, these species appear to be promising sources of various bioactive compounds that could be specifically used to treat certain diseases.

References

[1] Alexander, P.D., Alloway, B.J., Dourado, A. M. Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environ. Pollut.* 2006, 144, 736-745.

[2] Bursal, E., Köksal, E., Gülçin, I., Bilsel, G., Gören, A.C. Antioxidant activity and polyphenol content of cherry stem (*Cerasus avium* L.) determined by LC-MS/MS. *Food Res. Int.* 2013, 51, 66-74.

[3] Basgel S. and S. B. Erdemoglu. Determination of minerals and trace elements in some medicinal herbs and their infusions consumed in Turkey, *Science of the Total Environment,* 359 (2006) 82-89.

[4] Gülçin, ˙I., Beydemir, S. Phenolic compounds as antioxidants: Carbonic anhydrase isoenzymes inhibitors. *Mini Rev. Med. Chem*. 2013, 13, 408-430.

[5] Göçer, H., Gülçin, I. Caffeic acid phenethyl ester (CAPE): Correlation of structure and antioxidant properties. *Int. J. Food Sci. Nutr.* 2011, 62, 821-825.

[6] Jabeen S., M. Tahir Shah, S. Khan and M. Qasim Hayat. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan, *Journal of Medical Plants Reseach* 4 (2010) 559-566.

[7] Gowrishankar R., Kumar M., Menon V., Divi S. M., Saravanan M., Magudapathy P., Panigrahi B.K., Nair K.G., Venkataramaniah K. (2010). Trace element studies on *Tinospora cordifolia* (Menispermaceae), *Ocimu sanctum* (Lamiaceae), *Moringa oleifera* (Moringaceae), and *Phyllanthus niruri* (Euphorbiaceae) using PIXE. *Biol. Trace Elem. Res.* 133, 357363 10.1007/s12011-009-8439-1

[8] Kumar P.S., Mishra D., Ghosh G., Panda G.S. (2010). Medicinal uses and pharmacological properties of *Moringa oleifera*. *Int. J. Phytomed.* 2, 210 21610.5138/ijpm.2010.0975.0185.02017

[9] Khoddami, A., *et al.*, Technology for analysis of plant phenolic compounds. *Molecules* 18(2) 2013; 2328-75

[10] Khan, S.A., L. Khan, I. Hussain, K.B. Marwat and N. Akhtar. Profile of heavy metals in selected medicinal plants, Pakistan, *Journal of Weed Science Research*, 14 (2008) 101-110

[11] Karimi G., M.K. Hassanzadeh, A. Nili, Z. Khashayarmanesh, Z. Samiei, F. Nazari, M. Teimuri. Concentration and Health risk of heavy metals in tea samples marketed in Iran, *Pharmacology*, 3 (2008) 164-174.

[12] Louis H., Akakuru, O.U., Magu, T.O., Maitera O.N., Fidelis, T.T. and Bisong E.A., Comparative study of some heavy metal concentrations in coconut fluid and milk obtained from Taraba, Imo and Adamawa States, Nigeria.. *International Journal of Scientific and Research Publications*, Volume 7, Issue 3, March 2017,

[13] Louis, H., Tsware, T.T. Fidelis, J.O. Ogar, and E.A. Bisong. Formulation of alkyd- and Mechanical characterization. *The International Journal of Science* based polymer coating from treated seed of tsada *(Ximenia Americana)* seed oil: Physiocochemical *and Technoledge*, Vol. 3, Issue 8, 2015

[14] Louis H., Manassa., J.T. Barminas, T.T. Fidelis and E.A. Bisong. Screening of Persistant Organic Pollutant in surface water around Yola metropolis, Adamawa State. *International Journal of Scientific and Research Publications*, Volume 5, Issue 11, November 2015,

[15] Louis H., Gideon P.N., Nzei J.M., Munywoki J.M., Kwata F.G., Fidelis T., Phytodiversity, abundance and Importance of some useful plants at Mangrove swamp forest of Sobekiri, Rivers State, Nigeria *International Journal of Scientific and Research Publications*, Volume 7, Issue 3, March 2017

[16] Mann A., Phytochemical Constituents and Antimicrobial and Grain Protectant Activities of Clove Basil (*Ocimum gratissimum* L.) Grown in Nigeria. *International Journal of Plant Research*, 2(1):51-58, 2012

[17] Nwinyi O.C., Chinedu N.S., Ajani O.O., Ikpo C.O., Ogunniran K.O., Antibacterial effects of extrats of Ocimum gratissimum and piper guineense on Escherichia coli and Staphylococcus aureus. *African Journal of Food Science*, 3(3): 077-081, 2009

[18] Ozta¸skın, N., Çetinkaya, Y., Taslimi, P., Göksu, S., Gülçin, I. Antioxidant and acetylcholinesterase inhibition properties of novel bromophenol derivatives. *Bioorg. Chem.* 2015, 60, 49-57.

[19] *Telfairia Occidentalis*, 2009, Wikipedia, the free encyclopedia, retrieved on 4th July, http://en.Wikipedia.Org/Wiki/Telfairia_Occidentalis

[20] Sharma K.R., M. Agrawal, M.F., Marshall Heavy metals in vegetables collected from production and market sites of a tropical urban area of India, *Food and Chemical Toxicology*, 47 (2009) 583-591

[21] Säumel, I., Kotsyuk, I., Hölscher, M., Lenkereit, C., Weber, F., Kowarik, I. How healthy is urban horticulture in high traffic areas? Trace metal concentrations in vegetable crops from plantings within inner city neighbourhoods in Berlin, Germany. *Environ. Pollut.* 2012, 165, 124-132.

[22] Škerget, M., Kotnik, P., Hadolin, M. Hraš, A.R., Simonič, M., Knez, Z. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.* 2005, 89, 191-198.

[23] Yang, J., Guo, H., Ma, Y.,Wang, L.,Wei, D., Hua, L. Genotypic variations in the accumulation of Cd exhibited by different vegetables. J. Environ. Sci. China 2010, 22, 1246-1252

[24] 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. Environ. Manag.* 2009, 90, 1117-1122.

[25] Zhu, Y., Yu, H., Wang, J., Fang, W., Yuan, J., Yang, Z. Heavy metal accumulations of 24 asparagus bean cultivars grown in soil contaminated with Cd alone and with multiple metals (Cd, Pb, and Zn). *J. Agric. Food Chem.* 2007, 55, 1045-052.

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