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EVALUATION OF ESSENTIAL TRACE METALS ON THE THERAPEUTIC EFFICACY OF THE LEAVES OF NEWBOULDIA LAEVIS

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ABSTRACT

Trace elements are essential and play basic roles in a variety of processes essential for life. Their deficiency poses a serious problem that can affect the normal functioning of the immune system, especially in viral respiratory infections. Zinc and Copper are among the possible supportive treatments for COVID-19 infection due to their impact on the antiviral. Despite the increased use of the leaves of *Newbouldia laevis* for the treatment of a number of several diseases, the chemotherapeutic role of the inorganic constituents is still not clear. In this study fresh leaves samples of *Newbouldia laevis* were collected and prepared using 50 ml of a tri-acid mixture of HNO_3, $HClO_4$, and H_2SO_4 in the ratio of 25:4:2 respectively. The solution was then transferred into a 50 ml volumetric flask and made up to mark with distilled water. The levels of magnesium, iron, zinc, cobalt and copper were determined using Atomic Absorption Spectrometry. Concentrations of Zn were obtained and lie between 1.5306 to 1.5641 µg/g, and were found to be uniformly distributed in the five sampling sites. In addition, high concentrations of Fe in these leaves indicated the enhanced absorption of mineral iron and larger cryptoporosity, which implies retention and mobility of hydrophobic organic compounds. No risk to the consumers because the mean concentrations were below the permissible limit recommended by the health authority. The presence of trace elements could support the claims for the use of this plant to cure some of the reported ailments.

Keywords: Newbouldia laevis, Trace Metals, Atomic Absorption Spectrometry, Herbal therapy

INTRODUCTION

The use of medicinal plants is attracting a lot of interest from researchers and practitioners. For example, in West Africa medicinal plant recipes are mostly prepared as a decoction, infusion, maceration in water, ethanol and methanol or sauce. In addition, varying amounts and combinations of compounds that are present in different parts of plants like roots stems and leaves could explain the various therapeutic effects. The active ingredients of plant extracts are chemicals, like those of synthetic or purified drugs. Hence, understanding the bio-distribution of these secondary metabolites in roots, stems and leaves is key to gaining more insight into their pharmacological efficiency (Affo Dermane 2020). The *Newbouldia laevis (Bignoniaceae)*, popularly known as African Border tree is used traditionally for the treatment of several diseases, like cancer, diarrhea, dysentery etc. (Affo Dermane 2020; Usman 2007; Kolawole 2013; Ogunlana, 2008). For example, in Ivory Coast and Nigeria, stem bark decoctions of *Newbouldia laevis* are used for the treatment of convulsions in children. Also, the decoction of the roots with the roots of *Alstonia boonei, Jatropha curcas* are used for the treatment of epilepsy , the stem bark with clay and red pepper is used against pneumonia, fever, cold and cough (Idu M. and Omoruyi 2002).

Similarly, in Senegal, the bark of the tree, after pulping up to a paste is used for the treatment of Rheumatism; especially painful arthritis of the knees (Burkill 1985). In Nigeria, decoctions of the leaves and roots made from boiling are used as a febrifuge (Tor-anyiin, Sha'ato et al. 2003). Also, decoction of leaves alone is used against dental caries and sore eyes (Okeke 2003). Also in the treatment of breast tumors with bark and leaf decoctions is common in Ghana and Nigeria (Burkill 1997). Extracts of all parts of *Newbouldia laevis* have been shown to exhibit antimicrobial activity (Hounzangbe-Adote, Fouraste et al. 2005, V. Kuete 2007, E. O. Ogunlana 1975). Leaf and root extracts have been shown to possess anti-malarial properties (M. Gbeassor 1990, Kenneth Oben Eyong, Veronique Penlap Beng et al. 2006, Mann, Gbate et al. 2003). Fibrous stem bark when chewed is used in the treatment of abortifacient (Gbate et al. 2003). In addition, the phytochemical screening of the crude methanolic leaf extracts revealed the presence of flavonoids, tannins, terpenes, steroidal and cardiac glycosides, but alkaloids and Saponinwere found to be absent (Idu M. 2008, Gill 1992, Bouquet 1972).

These classes of compounds are known to show healing activity against numerous pathogens which explains its usage traditionally for the treatment of a wide array of illnesses. After the methanolic leaf extract was tested against some isolated microorganisms, namely *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella spp. and Candida albicans*; it showed inhibition to growth of the tested organisms, with the highest and lowest activity of 42.3 ± 1.5 mm and 14.3 ± 2.0 mm for *Klebsiella and Candida albicans* respectively. The presence of these organisms in the body may cause stomach discomfort, diarrhea, and dysentery. In addition, the extracts show marked higher activities. However, these activities are not comparable to those from most of the reference drugs (Usman 2007).

Although the effectiveness of medicinal plants for curative purposes is often accounted for in terms of their organic constituents, it has been established that there exists a relationship between the chelating of metals and some chemotherapeutic agents (Lamari 2008, L Hallberg 1966). It is also known that there is a significant role played by trace elements when treating various diseases (Lamari 2008). For example, the biochemical processes in the body make these trace elements to be involved in immunological and inflammatory reactions. Likewise, the keratinization and melanin formation are enzyme-dependent processes and

could be influenced by the deficiencies and excesses of trace elements (Hounzangbe-Adote, Fouraste et al. 2005, Wacewicz 2017). For example, selenium, zinc and copper are reported to be involved in the destruction of free radicals through cascading enzyme systems (Chan, Gerson et al. 1998).

The effect and influences of these elements on the administration of medicinal plant has received relatively little attention (Eyong, Folefoc et al. 2006). The roots absorb essential metals like Fe, Cu, Co (El-Rjoob 2007) and others from the soil and diffuse them up to the leaves which play the role of environmental monitors as they can reflect the compositional characteristics of the soil where they grow and the presence of toxicologically important heavy metals in the surrounding environment (Ahmed Y. A. 2010, Massadeh 2016). It has been reported that human system tends to retain Iron effectively. Hence only trace amounts from food sources are needed by the body on a daily basis. However, its deficiency can cause hypochromic anemia. On the other hand, Zn is an essential mineral that is involved in over twenty different enzymatic reactions in the body, ranging from the synthesis of protein and collagen to the production of cellular energy (Ahmed Y. A. 2010, Kankara 2021, Massadeh 2016). These body metabolisms will surely relieve conditions for some of the ailments for which *Newbouldia laevis* is reported to cure. There has been increasing research interest in the use of this plant, which brings about a rise in published articles every year (see Fig. 1).

To establish the elemental composition and efficacy of the *Newbouldia laevis* leaves and the possible medicinal uses of this plant, this study determined the levels of Co, Cu, Fe, Mg, and Zn in the leaves of the plant using Atomic Absorption Spectrometry (AAS).

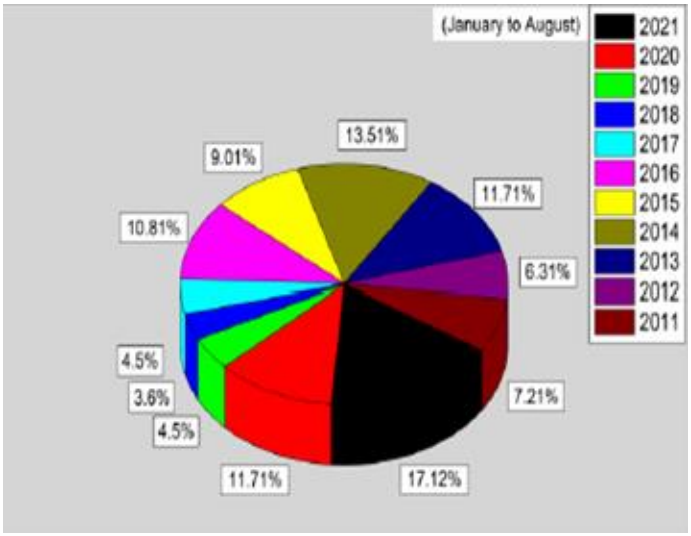

Figure 1: Number of publications of *Newbouldia laevis* based research. (2010-2021)

MATERIALS AND METHODS

Materials

All reagents and chemicals used were of analytical grade obtained from British Drug House (BDH) Poole. The concentration of the metals in the samples was determined using an Atomic Absorption Spectrophotometer (AAS), SHIMADZU model AA-6800 equipped with a hollow cathode lamp for each of the metals of interest. The lamp mode was BGCSR and the flame type consists of air/C_2H_2 fitting. The reference standards of Cu, Fe, Co, Mg, and Zn were manufactured by Scharlau chemia.

Method

Sample Collection and Pretreatment

Newbouldia laevis leaves were collected in polyethylene bags from five different selected points from Dakace Zaria, Nigeria (Fig. 2). The leaves were identified at the Botany department, Ahmadu Bello University, Zaria. Following the identification, the leaves were thoroughly washed with distilled water, freeze dried and ground to powder with agate at the material science laboratory of the Centre for Energy Research and Training, Ahmadu Bello University, Zaria. Subsequently, the samples were stored in a capped plastic container and labeled appropriately.

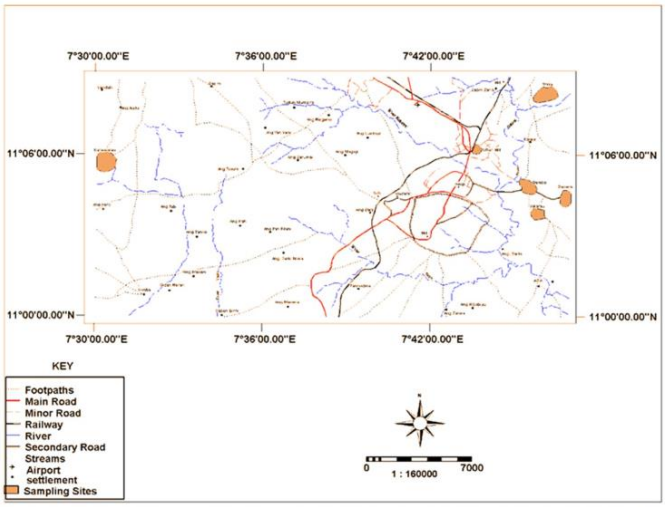

Figure 2: Zaria Metropolis showing the sampling sites
Source: Adapted and modified from Zaria Topo map sheet 102 S.W by GIS section Department of Geography, ABU Zaria

Sample Preparation and Analysis

Samples were digested as recommended by the Association of Official Analytical Chemists (AOAC), 1970. For each powdered sample, a portion (1g) was weighed, transferred into a beaker and digested using 50 ml of a tri-acid mixture of HNO_3, $HClO_4$, and H_2SO_4 in the ratio of 25:4:2 respectively, and then heated on a hot plate inside a fume cupboard at 100 ^oC. Heating was sustained until only about 2.5 ml of the sample was left in the beaker. The solution was then transferred into a 50 ml volumetric flask and made up to mark with distilled water. The digests obtained were then used for the analysis of metal concentration in the samples on the Atomic Absorption Spectrophotometer (AAS). For Zn and Cu concentrations the lamp current was about 10 mA with wave length of 213.9 nm and 324.8 nm respectively. In the case of Mg the wavelength was 285.2 nm and the lamp current was 8 mA. Both Fe and Co lamps had a current of 12.0 mA with wavelengths of 248.3 and 240.7 nm respectively. The metal concentrations for each sample were obtained from the calibration curve for each metal.

RESULTS AND DISCUSSION

Validation of the method

In order to estimate the analytical precision and accuracy and assure the proper quality of our results many requirements were considered during the analysis. For example, analyses of identical samples were performed. This duplication improves the quality of our results and can be used to assess the reliability. Furthermore, blank and standard solutions were used to calibrate the instruments. Also we used a set of standard calibration curves together with linear regression and standard deviations to measure the concentration of the heavy metals in soil and plant samples.

Concentrations (ppm) of the solution of each metal and their respective absorbance used in preparing the calibration curve in each case are presented in Table 1. The correlation coefficients of the regression relation for Cu, Fe, Co, Mg, and Zn in the standards were obtained as 0.9993, 0.9974, 0.9966, 0.9980 and 0.9901 respectively. The result of the standards shows the existence of the strong positive linear relationship between the concentration and the absorbance.

Table 1: Metal standards (ppm) and their respective absorbance used for the calibration curve

Cu	Abs.	Fe	Abs.	Co	Abs.	Mg	Abs.	Zn	Abs.
1	0.1714	1	0.1061	0.5	0.0033	0.2	0.2117	0.2	0.1013
2	0.2866	2	0.1443	1.0	0.0145	0.4	0.3345	0.4	0.2586
3	0.4194	3	0.1975	1.5	0.0235	0.6	0.4309	0.6	0.3381
4	0.5287	4	0.2588	2.0	0.0360	0.8	0.5267	0.8	0.4323
5	0.6360	5	0.3047	2.5	0.0426	0.0	0.0	1.0	0.6108

The results obtained for the analyzed elements namely Cu, Fe, Co, Mg and Zn in the leaves of *Newbouldia laevis* from the five sampling sites and their respective absorbance are shown in Tables 1 and 2. These elements are naturally occurring inorganic substances essential to our body in amounts of about <100 mg/day. They form vital components of biological structures and play a key role in a variety of the processes necessary for life through mediate biochemical reactions. The samples demonstrated high levels of Iron concentration in the range of 6.933 µg/g-10.1084 µg/g. On the other hand, Cu has a mean concentration of 0.4988 ± 0.014 with least concentration of 0.1960 µg/g at site D. The high concentration of Fe in these leaves indicates high absorption of mineral Iron by the plant roots at that sampling point compared with the other mineral elements of interest.

This is not surprising because it is by mass the most common element on Earth (Falah S. Al-Fartusie 2017). Thus, an imbalance in the optimum levels of trace elements may adversely affect biological processes and could be associated with many fatal diseases, such as cancers, etc. (Affo Dermane 2020). Hence, ingestion of leaves of the *Newbouldia laevis* could help reduce the nutritional deficiency caused by low levels of Fe, and also improving adequate amounts of hemoglobin to meet the body's oxygen transport needs.

Other reported Fe deficiencies that are related to inadequate oxygen supply to the body include fatigue, hair loss, and paleness in the hands and eyelids (Wintergerst 2007). It's a

fact that many studies indicated the importance of specific elements to have predictive significance in the early diagnosis and therapy evaluation of some diseases like epilepsy, dental caries, breast tumors etc. Hence, high concentration of Fe in our findings could buttresses the claims of the use of a decoction of the leaves to cure some of these ailments. Absorption of iron is influenced by the body stores, the chemical nature of Fe in the ingested food and by the variety of the dietary aspects responsible for the rise or decline in the availability of Fe for absorption (L Hallberg 1966, T. H. Bothwell 1980, M Gillooly 1983). However, supplementation of these elements much above the recommended dietary allowance (Press 1989) is highly discouraged (Ahmed Y. A. 2010).

Table 2: Concentrations of trace metals in *Newbouldia laevis* for five different sites

Element	Site 1	Site 2	Site 3	Site 4	Site 5	Mean Concentrations (µg/g ± SEM)
Cu	0.6964	0.6482	0.5185	0.1960	0.4349	0.4988±0.014
Fe	6.9334	8.3030	6.3858	9.5097	10.1084	8.2481±0.920
Co	0.0000	0.0359	0.2033	-0.0837	0.2871	0.0885±0.008
Mg	0.6188	0.0751	-0.0177	0.0671	-0.1599	0.1167±0.031
Zn	1.6541	1.6209	1.5206	1.6029	1.5559	1.5909±0.001

Our result shows that Co and Mg have the least mean concentration which lies between 0.001 to -0.0177µg/g. Co is regarded as an essential element and forms part of vitamin B12 which is required for red blood cell synthesis. It also has a substantial role in the formation of amino acids and neurotransmitters (Falah S. Al-Fartusie 2017). Toxic effects of Co include loss of body weight and depressed appetite. (Awofolu 2005) reported that vasodilatation, flushing and cardiomyopathy in humans and animals are due to the effect of a large amount of Cobalt. Therefore, the low levels of Co indicate poor erythrocyte production in the body however, the Co level in this work is within the safe limit. The compounds of heavy metals were reported to possess antimicrobial properties (Kenneth Oben Eyong, Veronique Penlap Beng et al. 2006). Therefore, this property might be attributed to the medicinal properties of *Newbouldia laevis*. On the other hand, alkaloids are found primarily in plants, well known alkaloids include morphine quinine. Therefore, the anti-malarial property shown by the leaves and roots extracts might be due to the alkaloid content of the plant. A significant relationship between the concentration of trace metals and sampling sites is observed. The correlation coefficient for different sites is above 0.9 in all the sites, which indicate a positive correlation as shown in Table 3.

Table 3: Correlation coefficients for all concentrations of the trace metals from the five sampling points

	site1	site 2	site 3	site 4	site 5
site1	1				
site 2	0.997	1			
site 3	0.994	0.999	1		
site 4	0.997	0.999	0.997	1	
site 5	0.992	0.998	0.997	0.998	1

Zinc and copper are an integral part of as many as forty metalloenzymes, including Cu/Zn superoxide dismutase with antioxidant and anti-inflammatory activity (Michalska 2016). The total antioxidant status of an individual is formed by enzymatic and non-enzymatic antioxidants in the body. So, the level of Zn in the *Newbouldia laevis* samples in this study could be used to evaluate the healing process associated with the plant. A uniform distribution in the concentration of Zn (1.5306 to 1.5641 µg/g) was observed from the five sampling sites. This is an indication that Zn plays a significant role in the healing properties of the *Newbouldia* leaves. It has been reported that Zn helps in protecting the liver from damage and promotes the rapid healing of wounds (Alan B. G. Lansdown, 2007; Lin, 2017).

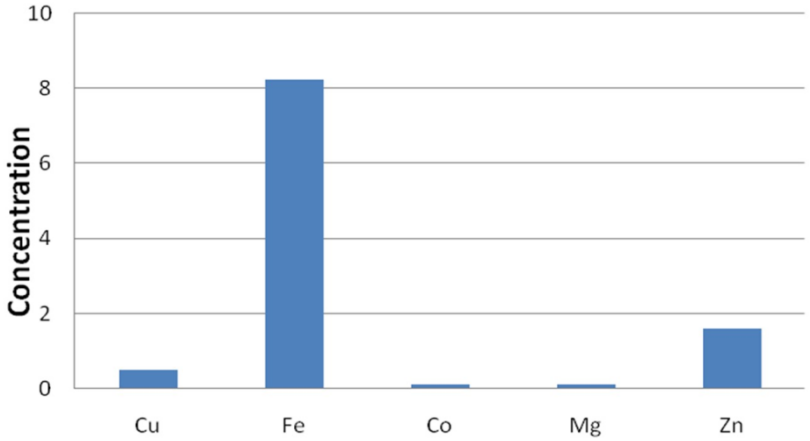

Figure 3: Histogram of the mean concentration of the metals in the samples

Zinc being an essential element that is needed to counter the effects of the Cd if taken together could be essential and important element for the users of this leave. Deficiency of Zn has been associated with a variety of diseases including sexual impotence (Shils 1988). The use of these leaves will therefore elevate Zn, Fe and other elements ingested by the patient and hence a potential cure for their deficiency. The toxicity of cadmium metal was reported earlier (Klevay 1975) to be counteracted by simultaneous ingestion of certain metals like cobalt, selenium and zinc. Furthermore, Zn could as well serve as a source of nutrients to the younger population of Africa, because the leaves are used in most of the African countries particularly Nigeria.

CONCLUSION

In conclusion, using AAS we have analyzed elemental composition in the leaves of the *Newbouldia laevis* and showed the distribution of essential trace elements namely Mg, Fe, Co, Cu, and Zn in five sampling sites. It can be deduced from our results that possible relief from diseases such as toxoplasmosis in women, weakness, and hair loss due to theinadequate amount of hemoglobin could be attributed to the high amount of Fe in the leaves. Also, the presence of Zn and Cu in the leaves may be the reason for the effective treatment of diarrhea and pneumonia in children. Based on our analysis and findings the present of these elements in the leaves of the *Newbouldia laevis* could support the claims for the use of this plant to cure some of the reported ailments.

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