Levels of trace metals content of crude ethanol leaf extract of globimetula oreophila (Hook. f) danser growing on *Azadirachta indica* using atomic absorption spectroscopy

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Abstract

Globimetula oreophila is a parasitic plant that is widely used in the treatment of various ailments including malaria, hypertension, cancer, diabetes, epilepsy, and as a diuretic agent. The present study is aimed at analyzing and documenting the level of trace and toxic metals in the crude ethanolic leaf extract of *G. oreophila*. After collection and authentication, the leaves were air-dried, mashed into powder, weighed, and extracted using 70% ethanol. Crude extract (0.5g) was digested with HNO_3: HCl (3:1); then heated to 200 ºC and analyzed for its metal content by atomic absorption spectroscopy (AAS). Fe had the highest concentration (32.73mg/kg), while Pb was not detected. The concentrations of Co, Cu, Ni, Zn, and Cd detected were 5.97, 10.8, 8.0,1, and 0.9 mg/kg respectively. The concentrations of Cd, Fe, and Ni were above the permissible limit of FAO/WHO.

Globimetula oreophila, medicinal plant, *Azadirachta indica*, elemental analysis, Atomic Absorption Spectroscopy.

Introduction

The relationship between humans and nature has been long and very complicated. Historical records, geography, time, and culture have not stopped humans from continuously searching, inquiring, and exploring the vast range of natural diversity for valuable products (Kong *et al.,* 2003). Historically speaking, humans have explored nature to satisfy two major needs i.e. food and herbs for alleviating pain and suffering (Kong *et al.,* 2003). Medicinal plants are considered a “backbone” of traditional medicine (TM), and about 3.3 billion people in less developed countries utilize medicinal plants regularly. These medicinal plants are considered rich resources of ingredients that can be used in drug development and synthesis (Davidson-Hunt, 2000). Inorganic contaminants such as heavy metals are often present in herbal medicine at various concentration levels (Saeed 2010; Hina *et al.,* 2011; Qing-hua *et al*., 2001). It is a scientific fact that prolonged ingestion of medicinal plants leads to the chronic accumulation(bioaccumaulation) of different elements which causes various health problems (WHO, 1992; Sharma *et al.,* 2009). Thus, the quality of such products has become a major safety concern (Igweze *et al.,* 2012). Most of these studies concluded that essential metals can also produce toxic effects when the metal intake is in high concentrations, whereas some non-essential metals are toxic even in very low concentrations.

Nigeria has rich vegetation and many of the plant species are used by some indigenous people for medicinal purposes (Adebayo and Krettli, 2011). *Globimetula oreophila*, Loranthaceae, popularly known as mistletoe (Adesina *et al.,* 2013). It is a parasitic plant that is widely used in the treatment of various ailments including malaria (Dauda *et al.,* 2016), hypertension, cancer, diabetes, epilepsy, and as a diuretic agent (Ojewole and Adewole, 2007). In recent years, several authors have pointed out the importance of elemental constituents in medicinal or edible plants which enhanced the awareness about trace elements in these plants (Wong *et al.,* 1993; Sharma *et al.,* 2009; Sheded *et al.,* 2006; Koe and Sari, 2009, Basgel and Erdemoglu, 2006; Ajasa *et al.,* 2004; Kanias and Loukis, 1987). The knowledge of the elemental composition of *G. oreophila* is therefore very important since it is used as medicine as well as food.

This study seeks to investigate the level of trace and toxic metals in the crude ethanol leaf extract of *G. oreophila*, compare the contents of *G. oreophila* leaves detected with tolerable maximum intake levels established by Dietary Reference Intakes (RDI), and to compare the values with those recommended by World Health Organization (WHO).

Materials and Methods

Chemicals and sample preparation

Solvents used in extraction, chemicals, and reagents used in this study were of pure analytical and trace metal grades. Trace metal grades 65 % HNO_3 and 37 % HCl were obtained from Fisher (Malaysia). Stock standard solutions for each metal, cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn), cobalt (Co), Copper (Cu,) and iron (Fe) with a concentration of 1000 ppm were supplied by Perkin Elmer (USA). Deionized water was used throughout the study. All glassware were soaked in 5% (v/v) HNO_3 overnight then rinsed with deionized water and dried using an oven before use.

Plant material and extraction

The plant sample of *Globimetula oreophila* growing on *Azadirachta indica* comprising the leaves and fruit was collected from Sokoto Metropolitan, Nigeria in July 2019. The plant sample was authenticated by a Taxonomist in the Department of Biological Sciences, Ahmadu Bello University Zaria by comparing it with a herbarium reference voucher specimen (Number ABU0886). After collection and authentication, the leaves were air-dried, mashed into powder, and weighed. The powder material (200 g) was extracted using 70% ethanol for 3 days with occasional shaking and filtered through muslin cloth and then through filter paper. The filtrate was concentrated using a rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) under reduced pressure at 40°C resulting in 20 g (10%) of dark green semisolid mass referred to as crude ethanol extract (CEE).

Elemental analysis

Crude extract (0.5 g) was weighed and placed in a 100-ml quick-fit round bottom flask. The sample was subjected to acid digestion methods, to determine the contents of Fe, Ni, Pb, Zn, Co, and Cd in *G. oreophila* leaves by Atomic Absorption Spectroscopy (AAS).

Method of Digestion (nitric-hydrochloric acid digestion 3:1)

To the sample, 9 mL of a freshly prepared acid mixture (3:1) of 65 % HNO3 was added, and 37 % HCl was added. Then, the mixture was boiled gently over a water bath (95 °C) for 4–5 h (or until the sample had completely dissolved) (Ang and Lee 2005). During the digestion procedures, the inner walls of the beakers were washed with 2 mL of deionized water to prevent the loss of the sample, and at the last part of the digestion processes, the sample was filtered with Whatman 42 (2.5-µm particle retention) filter paper. Then, a sufficient amount of deionized water was added to make the final volume up to 50 mL.

The solution was subjected to Flame Atomic Absorption Spectrometer (FAAS) (Shimadzu AA-670) to determine the different elements present (Ang and Lee 2005). The percentages of different elements in this sample were determined by the corresponding standard calibration curves obtained by using standard AR grade solutions of the elements, for example, Fe^{2+}, Co^{3+}, Cu^{3+}, Zn^{2+}, Ni^{3+}, Pb^{4+} and Cd^{2+}.

Results and Discussion

Analysis of seven elements namely Fe, Ni, Pb, Zn, Co, Cu, and Cd was performed on *G. oreophila* plant used in traditional medicine. The concentration of the metals and elements analyzed were recorded (Table I). The level of cadmium was found to be above permissible levels of FAO/WHO of 0.21 mg/kg in medicinal plant while Pb was not detected. The levels of the trace elements detected followed the order Fe > Co > Cu > Zn> Ni > Cd> Pb. The discussion of each element is presented as shown in the sequence of Table I. The elemental content in plants plays a crucial role in enhancing the body immune system against different diseases due to a definite correlation between mineral content in the human body with some disease conditions (Ceyik *et al.,* 2003).

Table 1: Distribution levels (mg/kg) of elemental contents in the triplicate samples of *G. oreophila* plant (mean values) and permissible limit (mg/kg) in edible plants and medicinal plants.

Element	leaves	FAO/WHO (1984)	WHO (2005)
Fe	32.73	20.0	-
Ni	5.97	1.63	10.0
Pb	0.00	0.43	10.0
Zn	8.01	27.40	50.0
Co	16.77	-	-
Cu	10.8	3.0	10.0
Cd	0.93	0.21	0.3

Zinc

The zinc content was found to be 8.01 mg/kg in *G. oreophila* leaves. Zinc is found throughout the entire body system, with half in the muscle tissue (Zevenhoven and Kilpinen, 2001). The established recommended daily amount (RDA) for Zn is 8 mg/day for women and 11 mg/day for men (Connie and Christine, 2009). The permissible limit of Zn in edible plants is 27.4 mg/kg (FAO/WHO, 1984) while that for medicinal plants is 50.0 mg/kg (WHO, 2005). After comparing the metal limit in *G. oreophila* plants with that proposed by FAO/WHO (1984), it is found that the Zn level was within the limit. According to Bowen (1966) and Allaway (1968), the range of Zn in agricultural products should be between 15 to 200 mg/kg.

The physiological activities of the plant influence the Zn absorption and interaction with many elements like Fe, Mn, and Cu, affects Zn uptake (Kandala *et al.,* 1974). About 270 enzymes depend on the activity of zinc (Zinpro, 2000), and its deficiency cause various physiological disorders in humans. Furthermore, Zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of Zinc for all life forms (FAO/WHO, 2001).

It plays a central role in the immune system, affecting several aspects of cellular and humoral immunity. Zinc is an extremely important part of insulin and it is known to improve the sensitivity of insulin in the management of diabetes (Shankar and Prasad, 1998).

Iron (Fe)

The level of Fe in *G. oreophila* leaves was 32.73 mg/kg, indicating a high solubility in the soil which might be attributed to the high availability of the soluble forms of this metal that can be absorbed. This value is above the permissible limit of 20 mg/kg (FAO/WHO, 1984). The limit of Fe in medicinal plants has not been established by WHO (1996). However, in Egypt, it ranges from 261-1239 mg/kg limits set in medicinal plants (Sheded *et al.*, 2006). The value obtained in this study is within the values stipulated by Egypt. Fe in the human body has three main functions. It is a part of haemoglobin and is responsible for oxygen transport, maintains a healthy immune system, and being a constituent of several enzymes, is responsible for energy production (Jasha and Petevino, 2016).

Copper

The copper content in leaf extract was 10.80 mg/kg which is above the permissible limit (3.00 ppm) set by FAO/WHO (1984) in edible plants. However, for medicinal plants, the WHO (2005) limit of 10.0 mg/kg was established for Cu.

Although in medicinal plants, permissible limits for Cu set by China and Singapore, were 20 mg/kg and 150 mg/kg respectively (WHO, 2005), values of 4 to 15 mg/kg have been cited in agricultural products (Bowen, 1966; Allaway, 1968).

Functional roles for Cu are some of the most important in human health are found in erythropoietin, myelin formation, modulation of catecholamine metabolism and antioxidant protection (Jasha and Petevino, 2016).

Cadmium

Cadmium is a nonessential element in foods and natural waters and accumulates principally in the kidneys, liver, and immune system (Heyes, 1997). The concentration of Cd in the studied plant was 0.93 mg/kg which is above the permissible limit (0.21 mg/kg) set by FAO (1984) for Cd in edible plants, but toxic effects have only been reported in man from the regular consumption of plants over 3 mg/kg (Ajasa *et al.,* 2004). The observed concentration is also higher than the permissible limit (0.3 ppm) for medicinal plants set for Cd by WHO, China, and Thailand.

Lead

The total lead content in most agricultural soils is within the range of 2 and 200 mg/kg (Yemene *et al.,* 2008), but in the present study, the lead concentrations in *G. oreophila* leaves was below the method detection limit. The permissible limit set by FAO/ WHO (1984) in edible plants was 0.43 mg/kg. The permissible limit for medicinal plants set by China, Malaysia, Thailand, and WHO was 10 mg/kg.

Cobalt

The concentration of Co in the studied plant was 16.77 mg/kg. Cobalt is essential for the healthy functioning of many plants and animals and it is often found in food. It is required for the normal functioning of the pancreas and for hemoglobin formation. Cobalt is an integral part of vitamin B12 which is an essential vitamin (Jabeen *et al.,* 2010). There are no established criteria for Coin medicinal plants.

Nickel

The concentration of Ni in *G. oreophila* plant was 5.97 mg/kg which is greater than the permissible limit (1.63 mg/kg) set by FAO/WHO (1984) for edible plants but lower that the limit (10.0 mg/kg) set for medicinal plants the WHO (2005). Nickel plays some roles in body functions including enzyme functions.

Table 2: Trace and essential elements of dietary allowance/intake in human adults.

Element	Recommended dietary allowance (RDA), ICMR, 2009	Recommended nutrient intake (RNI), FAO/WHO, 2004	Dietary reference intakes: recommended dietary allowance (RDA), USA
Fe	17.0 mg/d* and 21.0	9.1 mg/d*, 26.0	8.0 mg/d* , 18.0 mg/d**

	mg/d**	mg/d**	
Ni	-	-	1.0mg/d
Zn	10.0–12.0 mg/d	4.2–14.0 mg/d	11.0mg/d* and 8.0mg/d**
Co	-	-	-
Cu	1.35 mg/d	10.0 mg	900.0 μg/d

*For males. **For females. NE = not established, mg/g = milligram per day, and μg/d = microgram per day. Nutrient requirements and recommended dietary allowances for Indians. A Report of the expert group of the Indian Council of Medical Research (ICMR), 2009.

FAO/WHO: Food and Agriculture Organization of the United and World Health Organization, 2004.

Conclusion

The present study revealed that the *G. oreophila* plant is a good source of essential trace metals in appropriate quantities. However, the leaf of *Globimetula oreophila* collected from Sokoto may be unsafe for long-term use due to the high level of Fe, Ni, and Cd concentration. Therefore, special care must be taken into consideration during the administration of Mistletoe of Sokoto origin. It is also important to have good quality control practices for herbal medicines screening to protect consumers from toxicity.

Conflict of interest

There is no conflict of interest. No external financial support was obtained for this study

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