



# Quantitative Standards and Heavy Metals Concentration in the Leaves of *Isobertinia doka* from Selected Sites at Zaria, Northern Nigeria Guinea Savanna

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## Abstract

**Background:** *Isobertinia doka* is an evergreen hardwood tree with fast regenerating ability, widely distributed in northern Nigeria guinea savannah, exploited mainly for fuel wood and ethnomedicinal uses. **Objective:** This study evaluated the concentration of five heavy metals in *Isobertinia doka* leaves collected from three different activity area such as Botanical garden (A1), farming site at Zango (A2) and along high way (A3). **Method:** The quantitative data such as moisture content, total ash, acid insoluble ash, water soluble ash, ethanol and water extractives values, foaming index, swelling index, tannin content and bitterness value were determined using standard procedures. The concentration of Fe, Cd, Pb, Zn, and Cu were determined using atomic absorption spectrophotometer (AAS). **Results:** The quantitative data varied significantly among the three locations which were within the limit sets by Africa pharmacopoeia. The heavy metals detected in *I. doka* leaves from A1, A2, and A3 were in the same magnitude of Fe>Zn> Cu. Cd and Pb were not detected in all the three study site. The mean concentration of heavy metals detected at A1 were Fe (275.5 mg/kg), Zn (31.00 mg/kg), and Cu (8.30 mg/kg), A2 has a metal concentration of Fe (367.5 mg/kg), Zn (24.6 mg/kg) and Cu (5.60 mg/kg) while that of A3 were of Fe (539.1 mg/kg), Zn (97.00 mg/kg), and Cu (10.40 mg/kg). **Conclusions:** All the metal detected were within WHO/FDA normal permissible limit for heavy metal in herbal plant except for Fe and Zn in A3 which were above the limit. Analysis of variance shows variability between the three study sites with significant different at  $p \leq 0.05$  indicating the availability of some of these metal in *I. doka* leaves due to environmental pollution

**Keywords :** Heavy Metal; Quantitative; *Isobertinia Doka* ; Northern Nigeria

## 1. Introduction

*Isobertinia doka* belongs to the family Fabaceae (ceasalpinaceae). It exist as trees or shrubs measuring 10-20 m in height with a trunk of about 40-50 cm diameter. A compound shiny opposite leaves, the leaflets are arranged in three or four pairs. *I. doka* is an important component of Northern Nigeria guinea Savanah woodlands (Bello and Musa, 2016).

*I. doka* plant has been used in Nigeria traditional medicine for the treatment of diabetes, gastric ulcer, wounds, stomach pain, jaundice, arrow poison and cough (Magassouba et al. 2011, Abdu et al. 2016). Scientific investigations have confirmed its Phytochemical screening of the *I. doka* leave extract to contain saponins, flavonoids, alkaloids, terpenoids and tanins (Abdulkadir et al. 2011).

Study has showed scientific evidence that stem bark extract of *I. doka* enhanced sexuality in male Wistar rats (Minka et al. 2011). The Antioxidant Activity and Mineral Elements Profiles of *I. doka*

leaves has been reported (Abdulkadir et al. 2011). Brine shrimp lethality test of the root, stem bark and leaves extract of *I. doka* has also been reported by (Abdu et al. 2016).

Heavy metals are conventionally defined as elements with metallic property and atomic number greater than twenty which exist in nature. Emission from heavy traffic on roads contain lead (Pb), cadmium (Cd), zinc (Zn) and nickel (Ni), which are present in fuel as anti-knock agents (Atayese et al. 2009). Contamination of medicinal plants and herbal medicinal products by Heavy Metals has significant implications for human health when consumed (Moore and Modabberi, 2009, Odoh and Ajiboye 2019). Medicinal plants accumulate most of these heavy metals from the environment such as polluted soils, industrial and agricultural contamination through the food chain. Heavy metals contamination may disrupt the chemical nature of these plants thereby affecting the quality and efficacy of the natural plant. Exposure of heavy metal is increasing in many parts of the world, which is the lamentation of world health organisation and this has caused serious health issues such as carcinogenic effect, organs dysfunctions and death.

Heavy metal determination and quantitative standards are needed as criteria for quality control of medicinal plants to ensure safety and efficiency of herbal product (WHO 2011). *I. doka* has been used extensively in traditional medicines with only few scientific studies associated with its usage. This study is therefore necessary to assess the heavy metal concentration and quantitative standards in the leaves of *I. doka* from selected sites in Zaria, Northern Nigeria Guinea Savanna.

## 2. Materials and methodologies

### 2.1. Collection and identification of *Isobertinia doka*.

The fresh leaves of *I. doka* (plate I) were collected from three different sites located in Kaduna which include; Botanical garden of Department of Botany, Ahmadu Bello University, Zaria as A1, cultivation area in Zango as A2 and along road side Shika as A3 in June 2017. The plant was taxonomically authenticated by Mal. Namadi Sanusi with Voucher specimen number 022478 deposited at the Herbarium, Department of Botany, Ahmadu Bello University Zaria, Nigeria.



Plate 1: *I. doka* in natural habitat at Shika Zaria.



## 2.2 Preparation of *Isoberlinia doka* plant.

The collected fresh leaves, were washed in a clean water to removed sand and dirt's and air dried for one weeks. Pulverized into coarse powder with mortar and pestle and stored in cellophane bags at room temperature until required for experiment.

## 2.3 Sample treatment for heavy metal determination

For heavy metal analysis, 0.5 g of the sample was digested with 7.5 ml nitric acid and 2.5 ml hydrochloric acid and placed on a hot plate set at 100-170°C and remove when about to dry. On cooling, the digest was filtered into a beaker. Distilled water was added to the filtrate up to 30 ml.

## 2.4. Metal analysis:

The digest samples was analyzed for heavy metals (Pb, Cu, Fe, Cr and Zn) using atomic absorption spectrophotometer (AAS) of the model AA240FS.

## 2.5. Statistical analysis

One-way analysis of variance (ANOVA) test was carried out to examine the statistical significance of difference in the mean physicochemical and heavy metals between three different locations using at  $P \leq 0.05$  was considered statistically significant.

## 3. Results and Discussion

The quantitative standards determined at three different geographical locations in Zaria include moisture content, total ash, acid insoluble ash, water soluble ash, extractive value, foaming index, swelling index, tannins content, saponin content and bitterness value are very important standardization parameter according (WHO 2011). As showed in table 1.

Moisture content of the powdered leave drug was found to be  $5.22 \pm 0.02$  (% w/w) in A1,  $7.30 \pm 0.02$  (% w/w) in A2 and  $7.00 \pm 0.04$  (% w/w) in A3. Despite the slight differences in moisture contents A1, A2 and A3 they were still below the limit as stated by the (Africa pharmacopoeia 1980). Moisture content determination is important for crude drug because insufficient drying may lead to microbial contamination and possible enzymatic deterioration of active principles. This finding is similar to the earlier report of Bello et al. (2016) in the sense that they reported variations in physicochemical properties of medicinal plant found in different geographical locations. The results for total ash in A1, A2 and A3 were found to be 7.5 (% w/w), 3.08 (% w/w) and 5.51 (% w/w) respectively, which were within the normal limit stated for the family fabaceae. The amount of acid insoluble ash in A1 and A3 was 3.01 (% w/w) and 4.00 (% w/w) respectively while A2 value was very low 1.01(% w/w). According to (WHO 2011), the ash profiles varies among medicinal plants. The extractive values helps to determine the amount of active constituents in plants materials, the water extractive value of *I. doka* was found to be 9.80(% w/w) in A1, 8.85(% w/w) in A2 and 8.80 (% w/w) in A3, the ethanol extractive value (70%) was found to be 4.80 (% w/w) in A1, 4.70 (% w/w) in A2 and 4.70 (% w/w) in A3.the water extractive values in the three locations were higher than the ethanol extractive values which implies that the leaves contain enormous polar constituents. the swelling index of *I. doka* powdered leaves were found to be 170%, 178%, 176% at A1, A2 and A3 respectively this showed that the leaves has swelling ability which is due to an appreciable amount mucilage and hemicelluloses present. The foaming ability of an aqueous decoction of plant materials and their extract is measured in terms of a foaming index. The foaming index of *I. doka* determined was less than 100 in all the study location. This implied that the persistent foams that was observed when an aqueous decoction of *I. doka* leaves was shaken shows that the plant contain little saponins. Chemically, tannins are complex polyphenolic substances with astringent effect and therapeutic value.



The tannins content of *I. doka* in A1, A2 and A3 were 39.00 (% w/w), 30.22(% w/w) and 29.00 (% w/w) respectively which showed that *I. doka* leaves has astringency (WHO 2011).

The bitter properties of plant are determined by comparing the threshold bitter concentration of extract of the materials with that of a dilute solution of quinine hydrochloride (WHO 2011). The bitterness value of *I. doka* leaves was found to be 0.39% in all the study locations. This study showed that locations does not affect bitterness value of *I. doka* drugs table 1

The mean concentration of lead (Pb), Zinc (Zn), Cadmium (Cd), Iron (Fe) and Copper (Cu), obtained from *I. doka* leaves from the three different locations are summarized in Table 2. Only three of the metals (Fe, Zn and Cu) were detected in this study. This is in contrast with the findings of Bello et al. (2016) in which lead and cadmium were present. Cadmium and lead contamination in medicinal plant has serious adverse effects, this study has revealed that *I. doka* leaves collected from Zaria Nigeria are free from Cd and Pd which are safe for consumption and drugs production according to (WHO 2011). The heavy metal levels detected in A3 were significantly higher than the level observed in the reserved sites (A1) and farming site (A2).

The concentration of Fe in *I. doka* leaves were 275.5 mg/kg in A1, 367.5 mg/kg in A2 and 539.1 mg/kg in A3 which showed increase in concentration with increase in human activities, these values were below the permissible limit for medicinal plants except for A3. Iron (Fe) was found to be higher than any other metal in this study, which is in conformity with the findings (Ambi et al. 2017). High concentrations of iron in plants is due to the facts that natural soils contain significant levels of iron (Lawal et al. 2011). Plants uses iron for energy production processes and it helps to build human immunity so its deficiency in medicinal plants leads to adverse effect (Lenntech 2020, Ekere et al. 2018). Lead level in A1, A2 and A3 in *I. doka* were completely absent. Lead is a prime environmental pollutant and a probable human carcinogen. Exposure to high lead levels can severely damage the brain and kidneys and ultimately causes death (Martin and Grisword, 2009).

Zinc level in A1, A2 and A3 in *I. doka* were 31.00, 97.00 and 24.0 (mg/kg) respectively. Zinc is also the second most abundant metal obtained in this study. The level of zinc in *I. doka* analyzed in the three sites were within the permissible limits recommended by FAO/WHO (1978) in herbal medicine except Shika with a higher level, Zinc is an essential element in maintaining human health problems. The availability of copper (Cu) in *I. doka* showed increase in accumulation as 5.60 mg/kg in A2, 8.30 mg/kg in A1 and 10.40 mg/kg A3 and also within the safety limit. Cu is an important metal that regulates various metabolic process in human. This study has showed low bioaccumulation of cu in *I. doka* leaves which is in support of the findings of (Ekere et al. 2018). Copper contamination in herbal product that might lead to poisoning is not common.

**Table 1. Quantitative standard of Leaves of *I. doka* from Three Different Locations in % W/W.**

Parameter	A1: Mean $\pm$ SEM	A2:Mean $\pm$ SEM	A3:Mean $\pm$ SEM
Moisture Content	5.22 $\pm$ 0.02 <sup>b</sup>	7.30 $\pm$ 0.02 <sup>a</sup>	7.00 $\pm$ 0.04 <sup>a</sup>
Total Ash	7.50 $\pm$ 0.00 <sup>a</sup>	3.08 $\pm$ 0.00 <sup>c</sup>	5.51 $\pm$ 0.00 <sup>b</sup>
Acid Insoluble Ash	3.01 $\pm$ 0.01 <sup>c</sup>	1.01 $\pm$ 0.00 <sup>c</sup>	4.00 $\pm$ 0.00 <sup>b</sup>
Water Soluble Ash	6.00 $\pm$ 0.00 <sup>a</sup>	3.00 $\pm$ 0.00 <sup>b</sup>	1.50 $\pm$ 0.03 <sup>c</sup>
Water Extractive Value	9.80 $\pm$ 0.00 <sup>b</sup>	8.85 $\pm$ 0.03 <sup>b</sup>	8.80 $\pm$ 0.00 <sup>b</sup>
Ethanol Extractive Value	4.80 $\pm$ 0.00 <sup>a</sup>	4.70 $\pm$ 0.01 <sup>a</sup>	4.70 $\pm$ 0.02 <sup>a</sup>
Swelling index	170 $\pm$ 2.00 <sup>a</sup>	178.6 $\pm$ 1.01 <sup>a</sup>	176.7 $\pm$ 2.00 <sup>a</sup>
Foaming index	<100	<100	<100
Tannins content	39.00 $\pm$ 1.00 <sup>a</sup>	30.22 $\pm$ 2.00 <sup>a</sup>	29.00 $\pm$ 1.00 <sup>a</sup>
Bitterness value	0.39 $\pm$ 0.00 <sup>a</sup>	0.39 $\pm$ 0.01 <sup>a</sup>	0.39 $\pm$ 0.02 <sup>a</sup>

Values are expressed as mean  $\pm$  standard error of mean (SEM) of three replicates, Means on the same row followed by different superscripts are significantly different at P < 0.05.

**Table 2. Heavy Metals Concentrations *I. doka* from Three Different Sites in Zaria in Mg/kg.**

Treatment	A1	A2	A3	FAO/WHO (1976) limits	NORMAL RANGE
<b>Fe</b>	275.5 $\pm$ 0.01 <sup>c</sup>	367.5 $\pm$ 0.02 <sup>b</sup>	539.1 $\pm$ 0.02 <sup>c</sup>	48	400-500
<b>Zn</b>	31.00 $\pm$ 0.00 <sup>b</sup>	24.60 $\pm$ 0.00 <sup>c</sup>	97.00 $\pm$ 0.01 <sup>b</sup>	60	20-100
<b>Cd</b>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	1	<4
<b>Pb</b>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	2	0.5-30
<b>Cu</b>	8.30 $\pm$ 0.00 <sup>b</sup>	5.60 $\pm$ 0.00 <sup>b</sup>	10.40 $\pm$ 0.00 <sup>b</sup>	30	2.5

N=3, mean on the same row followed by different superscript are significantly different at p $\leq$ 0.05

#### 4. Conclusions

This study revealed that quantitative properties of the leaves of *I. doka* in different locations varied significantly, showing that plants, even though of the same species but from different sources may have different quantitative profiles and also has provided standards for correct identity and quality of this plants This study has shown that *I. doka* plants in different geographical locations accumulates different levels of heavy metals such as Fe, Zn and Cu due to human activities

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