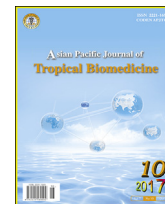




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## Phyto-metals screening of selected anti-diabetic herbs and infused concoctions

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## ABSTRACT

**Objective:** To determine the levels of some selected heavy metals in both the selected anti-diabetic herbal plants and infused concoctions for diabetes treatment.**Methods:** Ten anti-diabetic plant samples: pawpaw leaves (*Carica papaya*), bitter melon leaves (*Momordica charantia*), holy basil leaves (*Ocimum sanctum*), bitter leaf (*Vernonia amygdalina*), ginger rhizome (*Zingiber officinale*), garlic (*Allium sativum*), African red pepper fruits (*Capsicum frutescens*), negro pepper grain (*Xylopiya aethiopica*), cashew leaves (*Anacardium occidentale*) and onion bulb (*Allium cepa*) were evaluated for heavy metals. These were digested using standard methods and analyzed for manganese, copper, nickel, chromium, zinc, cadmium and lead using atomic absorption spectrophotometer. The infused concoctions (I and II) prepared from these medicinal herbs administered to diabetic patients were also analyzed for these heavy metals. Concoction I contained all the plants and honey with the exception of *Momordica charantia* and *Ocimum sanctum* which constituted concoction II with water only. The data obtained were subject to descriptive (mean and standard deviation) and inferential (ANOVA and DMRT) statistics.**Results:** Chromium and nickel levels were below detection limits in concoction I while manganese [(0.11 ± 0.01) µg/g] and zinc [(0.09 ± 0.01) µg/g] were detected in concoction II. Honey contained manganese [(0.10 ± 0.01) µg/g] and nickel [(0.70 ± 0.01) µg/g]. The anti-diabetic medicinal herbs and infused concoctions (I and II) were observed to contain heavy metals below the compared limits.**Conclusions:** The study thus shows that the herbs and concoctions are safe from the heavy metals considered. However, right dosage of the anti-diabetic concoctions should always be considered to prevent possible chronic side effects from bio-accumulation of heavy metals.

## 1. Introduction

Many of the pharmaceuticals currently available to physician have a long history of usage as herbal remedies, including aspirin, quinine and opium. Most plants produce chemical compounds as part of their normal metabolic activities. Some

plants having been found medicinal are useful for healing as well as for curing human diseases because of the presence of these phytochemical constituents [1]. The chemical compounds from plants are referred to as phytochemicals and are non-nutritive plant chemicals with ill-health protective or preventive characteristics. These chemicals are what plants produced for defense mechanisms but they recently demonstrated that they can help humans and animals fight against ill-health [2]. Medicinal derivative plants are part of traditional health care in most parts of the world thousands of years ago [3]. Medicinal herbs which are confirmed from studies to have anti-diabetic and related beneficial effects are used diabetes treatment [4]. Diabetes mellitus is described as increasing in

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plasma glucose concentrations owing to inadequate insulin and insulin resistance, or collectively, resulting in metabolic aberrations of carbohydrates, lipids and proteins [5]. Diabetes mellitus is also described to be an endocrine and metabolic disorder that exhibits significant menace in the millennium [6]. The dominantly detected features of diabetes mellitus are hyperglycemia, hypoinsulinemia and dyslipidemia. The disorders brought about by diabetes are said to be currently controlled by diet, exercise, insulin substitute therapy as well as herbal hypoglycemic agents [7]. The figures obtained from the world ethnobotanists report showed that nearly 800 medicinal plants could be used to regulate diabetes mellitus [8]. Medicinal herbs were observed to be reservoirs of natural products which have possible anti-diabetic effects [9]. Some anti-diabetic plants were confirmed useful traditionally because of their phytochemical compositions and pharmacological actions. The anti-diabetic influence could be traced to the presence of bio-active constituents of medicinal value and different chemical elements which include alkaloids, sterols, essential oils and triterpenes [10].

The different types of compounds (alkaloids, tannins, saponins, flavonoids and glycosides, phenols) determined at varying levels were suggested to be responsible for anti-diabetic activities in several plants screened [9,11,12]. The report of a research by Aderibigebe and Emudianughe [13] showed anti-diabetic activity of aqueous leaves of some medicinal herbs. The hypoglycemic effect of the tested plant was suggested to be by decreasing in intestinal absorption of glucose. Anti-hyperglycemic activity of aqueous stem bark extract of a medicinal herbal was studied [14] with the extract tested on diabetic rats. There was an observation of a significant decrease in blood glucose level. The leaf extract of another herbal medicine was reported for anti-diabetic efficacy in rats [15]. High percentage of countries in Africa depends on traditional medicine, which the World Health Organization (WHO) had recognized since 1978 to have probable usefulness in primary health care. This prompted WHO to devise a guideline framework for the efficacy and safety evaluation of herbal medicine [16,17]. Quite a number of people resorted to herbal medicine as a substitute for conventional remedy with the view that herbal medicine is a natural remedy for diabetes [18]. Conventional drugs had been studied to establish glucose homeostasis by either promoting insulin secretion or uptaking glucose through muscle cells [19]. The conventional drugs are known to have clinical health side effects [20]. Herbal medicines are relatively more cost-effective than synthetic drugs. One of the identified issues with herbal preparation is heavy metal toxicity [21] which often leads to heavy metal poisoning risk to the patients [22-24]. Most of the side effects from herbal medicines high consumption were associated to the poor quality of herbal drugs from the raw ingredients contamination and augmentation of heavy metals to boost effectiveness of the drugs.

Consequently, phytochemicals are subordinate metabolites which are mostly malignant molecules with consequential toxicity when used [25]. Herbal medicines had thereafter come under scrutiny due to their perceived long term toxicity possible from heavy metals accumulation in different parts during growing, processing and handling [26,27]. The levels of harmful heavy metals accumulated depend on growing stage, plant species, category of ion concentration among other factors [28]. Causes of the toxicities of various plants including

herbals are attributable to their chemical and mineral contents. This thus necessitates phyto-screening for quality control [29,30], as metal intake by human could be toxic at higher concentrations while non-essential metals are toxic even though at low concentrations [31]. These metals could be present in various concentrations in the extracts. No human part or system are exempted from suffering adverse effects that are possible when heavy metals get accumulated by human [32,33]. The research thus determined the levels of some selected heavy metals in both the selected anti-diabetic herbal plants and infused concoctions for diabetes treatment.

## 2. Materials and methods

### 2.1. Sample collection, preservation and preparation

All the herbal plants for the treatment of diabetes mellitus used during the course of this research were purchased from different locations in Ibadan, Oyo State: eight herbals (green pawpaw leaves, bitter lemon, holy basil leaves, bitter leaves, African red pepper, Negro pepper grain, cashew stem-bark, bulbs of onions) from Ologun-eru and two (garlic and ginger) from Eleyele. All the plants were thoroughly washed in distilled water and air-dried before they were transferred into separate zip-black bags prior to digestion and analysis. Grinding was done using pre-cleaned pestle and mortar.

### 2.2. Preparation of local anti-diabetic concoction

#### 2.2.1. Materials and preparation of concoction I

The selected medicinal plant materials were 7 green pawpaw leaves, a measure (medium size container) bitter lemon, 10 holy basil leaves, 10 bitter leaves, 10 pods of African red pepper, 15 pieces of negro pepper grain, 1 kg bucket of cashew stem-bark, 5 bulbs of onions, 10 bulbs of garlic, 10 pieces of ginger, 15 L of borehole sourced water and two bottles of honey. The list of the herbal plants used is as presented in Table 1. The collected herbal plants were thoroughly washed with borehole water, properly packed in a pre-cleaned pot: negro pepper grain was first placed in the pot, followed by garlic, ginger, onion, African red pepper, cashew stem-bark, green pawpaw leaves and bitter leaves. Water was then added to the pot level and boiled for 40 min and allowed to be cooled for 24 h. Two liters of honey was then added. The diabetic patients would be expected to take 5 cL glass cup of this concoction I three times a day for two months. These preparations were based on local knowledge.

#### 2.2.2. Materials and preparation of concoction II

Bitter melon, holy basil leaves and borehole sourced water. Ten pieces of these two herbal plants were thoroughly washed with water and their therapeutic contents were extracted with water by infusion, paying little attention to the precise quantity but making the prepared concoction as thick and concentrated as possible. The diabetic patients would be expected to take 1 glass cup of this concoction II three times a day for two months. These preparations were based on local knowledge.

### 2.3. Methods of extraction: infusion

The extraction method involved separation of active portion of all the herbal plants from the inactive/inert components. For

the dried samples: the collected samples were washed, air-dried and liquid infused. Concoction II was made from both *Momordica charantia* and *O. sanctum* infused with non-boiled water. Concoction I containing the remaining 8 medicinal herbs were boiled with water for infusion, left to be cooled for 24 h and then 2 L of honey was added.

#### 2.4. Digestion of samples

A 1 g ground sample was weighed in triplicate into 3 conical flasks followed by addition of 10 mL HNO<sub>3</sub> and this was repeated for each medicinal plant. From Concoction I, 20 mL was measured in triplicate in 3 conical flasks, followed by addition of 10 mL HNO<sub>3</sub> for digestion and repeated for Concoction II. For honey sample, 20 mL of it was added to 80 mL of distilled water, followed by addition of 10 mL HNO<sub>3</sub> for digestion. The solutions were heated on a hot plate in fume cupboard until the dense brown fumes of HNO<sub>3</sub> faded. The digested samples were left to be cooled after they were filtered and made up to 100 mL with distilled water. The samples were then analyzed with atomic absorption spectrophotometer (Buck Scientific 200, UK).

#### 2.5. Statistical analysis

All statistical analyses were carried out using the Statistical Package for Social Scientists (SPSS 19.0) Software. One-way ANOVA and Pearson's correlation index were used to test for significant differences (95% confidence level).

### 3. Results

The analytical results of the selected heavy metals are as presented in Table 2. The major heavy metals detected were manganese (Mn), copper (Cu) and zinc (Zn). Other metals:

chromium (Cr) (except in the bitter leaf), cadmium (Cd), plumbum (Pb) and nickel (Ni) were detected below the detection limits of the instrument. As some trace essential elements have significant useful functions in human body with their roles in fighting against diabetes yet to be well understood, others [Mn, Zn, Cr and vanadium (V)] have important roles in metabolism and insulin action [34].

#### 3.1. Essential trace elements

From Table 2, levels of Mn ranged from (0.10 ± 0.01) µg/g in *A. cepa* and *A. occidentale* to (4.20 ± 0.05) µg/g in *Z. officinale*. There was significant difference in the concentration of Mn in all the selected anti-diabetic plants ( $P < 0.05$ ). Concentrations of Zn ranged from (0.10 ± 0.01) µg/g in *X. aethiopicum* to (0.52 ± 0.02) µg/g in *A. sativum*. Its level was significantly different in all the anti-diabetic plants studied and highest in both *A. sativum* and *V. Amygdalina* ( $P < 0.05$ ). Levels of Cu in all the anti-diabetic plants ranged from (0.07 ± 0.01) µg/g in *Z. officinale* to (0.16 ± 0.02) µg/g in *O. sanctum*. The level was not significantly different in all the medicinal herbs analyzed ( $P < 0.05$ ). Cr was only present (0.15 ± 0.00) µg/g in *V. amygdalina*.

#### 3.2. Toxic elements

Cd and Pb were detected below the detection limits of the instrument for all the selected anti-diabetic plants (Table 2).

#### 3.3. Levels of both essential trace and toxic elements in concoctions I and II

Mean concentration of Mn [(0.72 ± 0.01) µg/g] in concoction I (Table 3) was lower than the level [(0.80 ± 0.01) µg/g] detected

**Table 1**

Selected plants and the plants parts.

English name	Botanical name	Local name	Plant part used
Pawpaw	<i>Carica papaya</i>	Ibepe (Yoruba)	Leaves
Bitter melon	<i>Momordica charantia</i>	Ejinrin (Yoruba)	Leaves
Holy Basil	<i>Ocimum sanctum</i> ( <i>O. sanctum</i> )	Efirin (Yoruba)	Leaves
Bitter leaf	<i>Vernonia amygdalina</i> ( <i>V. amygdalina</i> )	Ewuro (Yoruba)	Leaves
Ginger	<i>Zingiber officinale</i> ( <i>Z. officinale</i> )	Ataale (Yoruba)	Rhizome
Garlic	<i>Allium sativum</i> ( <i>A. sativum</i> )	–	Bulb
Africa red pepper	<i>Capsicum frutescens</i>	Atare (Yoruba)	Fruit
Negro pepper grain	<i>Xylopiya aethiopicum</i> ( <i>X. aethiopicum</i> )	Iyere (Yoruba)	Fruit
Cashew	<i>Anacardium occidentale</i> ( <i>A. occidentale</i> )	Kaju (Yoruba)	Bark
Onion	<i>Allium cepa</i> ( <i>A. cepa</i> )	Alubosa (Yoruba)	Onion bulb

**Table 2**

Distribution levels of some heavy metals in selected anti-diabetic plants (mean ± SD,  $n = 3$ ) (µg/g).

Samples	Mn	Cu	Zn	Cr	Cd	Pb	Ni
<i>Carica papaya</i>	0.60 ± 0.01 <sup>b</sup>	0.10 ± 0.14 <sup>a</sup>	0.26 ± 0.01 <sup>d</sup>	< DL	< DL	< DL	< DL
<i>Momordica charantia</i>	1.00 ± 0.01 <sup>d</sup>	0.08 ± 0.01 <sup>a</sup>	0.41 ± 0.01 <sup>e</sup>	< DL	< DL	< DL	< DL
<i>O. sanctum</i>	0.55 ± 0.41 <sup>b</sup>	0.16 ± 0.02 <sup>a</sup>	0.40 ± 0.01 <sup>c</sup>	< DL	< DL	< DL	< DL
<i>V. amygdalina</i>	0.80 ± 0.01 <sup>c</sup>	0.12 ± 0.01 <sup>a</sup>	0.31 ± 0.01 <sup>f</sup>	0.15 ± 0.00 <sup>a</sup>	< DL	< DL	< DL
<i>Z. officinale</i>	4.20 ± 0.05 <sup>f</sup>	0.07 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>c</sup>	< DL	< DL	< DL	< DL
<i>A. sativum</i>	1.24 ± 0.01 <sup>c</sup>	0.10 ± 0.01 <sup>a</sup>	0.52 ± 0.02 <sup>f</sup>	< DL	< DL	< DL	< DL
<i>Capsicum frutescens</i>	0.31 ± 0.01 <sup>a</sup>	0.08 ± 0.00 <sup>a</sup>	0.24 ± 0.01 <sup>c</sup>	< DL	< DL	< DL	< DL
<i>X. aethiopicum</i>	0.61 ± 0.03 <sup>b</sup>	0.13 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	< DL	< DL	< DL	< DL
<i>A. occidentale</i>	0.10 ± 0.01 <sup>a</sup>	0.08 ± 0.01 <sup>a</sup>	0.22 ± 0.01 <sup>b</sup>	< DL	< DL	< DL	< DL
<i>A. cepa</i>	0.10 ± 0.01 <sup>a</sup>	0.08 ± 0.01 <sup>a</sup>	0.22 ± 0.01 <sup>b</sup>	< DL	< DL	< DL	< DL

Values down the column with the same superscript are significantly different ( $P < 0.05$ ). < DL = less than the detection limit.

**Table 3**

Distribution levels of some heavy metals in anti-diabetic concoctions I and II (mean  $\pm$  SD,  $n = 3$ ) ( $\mu\text{g/g}$ ).

Parameter	Concoction I	Concoction II	FAO/WHO [35]
Mn	0.72 $\pm$ 0.01 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	44.6–339
Cu	0.06 $\pm$ 0.01 <sup>a</sup>	< DL	0.14–0.48
Zn	0.24 $\pm$ 0.01 <sup>b</sup>	0.09 $\pm$ 0.01 <sup>a</sup>	27.40
Cr	0.02 $\pm$ 0.04 <sup>a</sup>	< DL	2.00
Cd	0.01 $\pm$ 0.02 <sup>a</sup>	< DL	0.30
Pb	< DL	< DL	10.00
Ni	< DL	< DL	1.63

Values down the column with the same superscript are significantly different ( $P < 0.05$ ). < DL = Less than the detection limit.

in *V. amygdalina* but higher than the levels [(0.60  $\pm$  0.01)  $\mu\text{g/g}$ ] and [(0.61  $\pm$  0.03)  $\mu\text{g/g}$ ] detected in *Carica papaya* and *X. aethiopica* (Table 2). The level of Mn detected in concoction II was (0.11  $\pm$  0.01)  $\mu\text{g/g}$  (Table 3) while that was detected in both *A. occidentale* and *A. cepa* was (0.10  $\pm$  0.01)  $\mu\text{g/g}$  (Table 2). Concoction I [(0.72  $\pm$  0.01)  $\mu\text{g/g}$ ] had higher Mn concentrations than concoction II [(0.11  $\pm$  0.01)  $\mu\text{g/g}$ ] (Table 3). Mn level was significantly different in both concoctions I and II ( $P < 0.05$ ). Level of Cu [(0.06  $\pm$  0.01)  $\mu\text{g/g}$ ] in concoction I was lower than the FAO/WHO standard (Table 3). The mean Zn concentration in concoction I [(0.24  $\pm$  0.01)  $\mu\text{g/g}$ ] was significantly higher than the level in concoction II [(0.09  $\pm$  0.01)  $\mu\text{g/g}$ ] ( $P < 0.05$ ). The level of Zn was the same as detected in the fruit of African red pepper (*Capsicum frutescens*) but lower than in other herbs (Table 2). Concentrations of both Cr [(0.02  $\pm$  0.04)  $\mu\text{g/g}$ ] and Cd [(0.01  $\pm$  0.02)  $\mu\text{g/g}$ ] in concoction I were respectively greater than values in concoction II (Table 3) and other medicinal herbs where they were below the detection limits (Table 2). Both Pb and Ni were below the detection limits in concoctions I, II (Table 2) and other medicinal herbs (Table 2). Levels of all the analyzed heavy and toxic metals were below the detection limits of the instrument except Mn [(0.10  $\pm$  0.01)  $\mu\text{g/g}$ ] in honey.

## 4. Discussion

### 4.1. Essential trace elements

The highest concentration of Mn detected is lower than values reported in previous studies carried out on medicinal plants [34–36]. However, the range of the results reported is similar to the findings of Moses *et al.* [37]. Manganese deficiency has been observed in various species of animals with the signs of impaired growth, impaired reproductive function, impaired glucose tolerance and alterations in carbohydrate and lipid metabolism. Furthermore, manganese deficiency interferes with normal skeletal development in various animal species [38–40]. Manganese is known to be an enzyme activator of the insulin metabolism [40]. The highest concentration of Zn [(0.52  $\pm$  0.02)  $\mu\text{g/g}$ ] in the bulb of garlic (*A. sativum*) in this study was lower than other previous studies [34–37].

Zn is known to assist in the regulation of insulin levels in the blood and has been reported to improve the sensitivity of insulin in the management of diabetes [41]. Zn is an essential component of enzymatic and redox reactions in human body [42]. The trend for Zn is similar to that was reported in herbal plants in the

Northern Nigeria [43] but exceeded the range reported by Ajasa *et al.* [44] for medicinal plants in South-western Nigeria. Copper is equally an essential component of enzymatic and redox reactions in human body [42]. The highest concentration of Cu detected [(0.16  $\pm$  0.00)  $\mu\text{g/g}$ ] in *O. sanctum* was lower than the values previously reported [34–37]. Both Zn and Cu are moderately extractable according to Pytlakowska *et al.* [45] that categorized elements into three groups with slight changes from the groups reported by Natesan and Ranganathan [46]. The level of Cr concentration was lower than the values Djama *et al.* [34] and Moses *et al.* [37] reported but similar to the report of Olujimi *et al.* [35].

Cr potentiates the action of insulin *in vivo* and *in vitro* [47–49]. Its deficiency in total parenteral nutrition patients impairs glucose utilization and raises insulin requirements. Thus it has been hypothesized that poor Cr status is a factor contributing to the incidence of impaired glucose tolerance and Type II diabetes [50]. Cr supplement (particularly chromium picolinate) use has increased in popularity as a result of reports that Cr potentiates the action of insulin and reduces hyperglycaemia and hyperlipidemia [51]. Concentration of Ni was lower than the detection limit of the instrument and the levels previously reported [34,35,37].

### 4.2. Toxic elements

Levels of Cd and Pb were lower than the levels reported by Moses *et al.* [37] and Olujimi *et al.* [35]. The most recognized toxic environmental pollutant is Pb and it accumulates in the skeleton especially in the bone marrow; it is a neurotoxin and causes behavioral abnormalities, retarding intelligence and mental development [52].

### 4.3. Levels of both essential trace and toxic elements in concoctions I and II

The determined levels of Cu [(0.06  $\pm$  0.01)  $\mu\text{g/g}$ ] and Cr [(0.02  $\pm$  0.04)  $\mu\text{g/g}$ ] respectively conformed to and higher than the mean range values (0.038–0.061  $\mu\text{g/g}$ ) and (0.010–0.015  $\mu\text{g/g}$ ) determined by Nathan *et al.* [27] in some medicinal plants. The levels of all the metals determined in this study were lower than the heavy metals daily intake stipulated by FAO/WHO [35] except the range of levels of Cu [from (0.07  $\pm$  0.01)  $\mu\text{g/g}$  in *Z. officinale* to (0.16  $\pm$  0.02)  $\mu\text{g/g}$  in *O. sanctum*] which was within the FAO/WHO limits (0.14–0.48)  $\mu\text{g/g}$ . This indicated that levels of these metals would hardly pose clinical threats to the health of the consumers of both concoctions I and II. However, chronic effects of administration ought to be taken into consideration because of the length of time (2 months) prescribed for the consumers.

In conclusion, from this study, it could be observed that all the anti-diabetic medicinal herbs selected were safe from heavy and trace metals considered. Their levels varied significantly in all the samples, and the risk is that their availability will also vary depending on the solvent (honey and water) used in preparing the concoctions. Right dosage of these concoctions should always be considered to prevent chronic side effects from the toxic metals gradual bio-accumulation. There should also be intervals in the consumption of these concoctions to create gaps for the right dosage. The medicinal plants source for the treatment of ailment including diabetes must not be taken from heavy



metal contaminated areas to avoid their uptake by the plants because migration of these contaminants into non-contaminated areas (or leaching through the soil and spreading of heavy metals contaminated sewage sludge) are a few examples of events contributing to contamination of the ecosystem.

### Conflict of interest statement

The authors declare that they have no conflict of interest.

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