FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Investigation of heavy metals in selected local spices consumed in Ethiopia

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Abstract: In Ethiopia, Natural food spices such as sweet basil (Ocimum basilicum L.), Black Cumen (Nigella sativa) and Tenadam (Ruta chalepensis) are the most commonly used spices to improve its colour, aroma, palatability and acceptability of food. They contain a significant quantity of trace metals. These trace metals in spices and medicinal plants play an important role in living cells while other metals are harmful. Knowing the composition of trace metals in foods is important because of their essential or toxic in human health. This study focuses on the determination of heavy trace metals (Zn, Cu, Fe, Cd, and Pb) from Ocimum basilicum L., Nigella sativa, and Ruta chalepensis cultivated in Ethiopia North Gondar administrative Zone particularly, Koladiba, Takusa, and Chuahit were determined using FAAS. The result showed that the concentration of Zn, Cu, Fe, Cd, and Pb in three cultivars of Nigella sativa was 9.07 ± 0.055, 2.83 ± 0.021, 5.55 ± 0.019, 0.80 ± 0.087 and 5.23 ± 0.028 mg/100 g, respectively. In Ocimum basilicum L. was 8.61 ± 0.067, 3.58 ± 0.021, 5.55 ± 0.019, 0.606 ± 0.0015, and 3.33 ± 0.0082 mg/100, respectively. Similarly in Ruta chalepensis were 9.38 ± 0.0705, 9.38 ± 0.021, 6.66 ± 0.033, 0.707 ± 0.0017 and 2.85 ± 0.0014 mg/100, respectively. In this work, Nigella sativa, Ocimum basilicum, and Ruta chalepensis are good sources of Zn, Cu, Fe, and the number of toxic metals Cd and Pb are below the permissible limit as compared to the value reported in WHO. Therefore, a non-carcinogenic health risk to the consumer associated with the consumption of this spices preparation marketed in study areas.

ABOUT THE AUTHOR

I am Fikirte Zewdu (MSc) working at Department of Chemistry, College of Natural and Computational Sciences, University of Gondar, Gondar, Ethiopia. Our group is mainly engaged in both basic and applied pieces of research. Our research article is an Investigation of Heavy Metals in Selected Local Spices Consumed in Ethiopia.

PUBLIC INTEREST STATEMENT

Natural food spices such as the Nigella sativa, Ocimum basilicum L. and Ruta chalepensis contain a significant quantity of trace metals. These trace metals in spices and medicinal plants play an important role in living cells. Knowing the composition of trace metals in foods is important because of their essential or toxic in human health. Heavy metals are non-essential for our body even at a low amount. Besides, dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. The addition of spices that may be contaminated with trace and heavy metals to food as a habit may result in the accumulation of these metals in human organs. The accumulation of heavy metals can have middle-term and long term health risks, and strict periodic surveillance of these contaminants is therefore advisable.
Subject: Agriculture & Environmental Sciences; Biotechnology; Food Additives & Ingredients; Food Chemistry

Keywords: Nigella sativa; Ocimum basilicum L.; Ruta chalepensis; heavy metals; concentration; FAAS

1. Introduction
Spices are dried parts of plants, which have been used as dietary components of food often to improve its colour, aroma, palatability and acceptability of food. Most of these are fragrant, aromatic and pungent (Mubeen et al., 2009). Many spices have antimicrobial properties (Moses and Uwash, 2015). This may explain why spices are more commonly used in warmer climates, which have more infectious diseases, and why the use of spices is prominent in meat, which is particularly susceptible to spoiling. Spices may have other uses such as medicinal, religious ceremony, cosmetics or perfumes production (Moses and Uwash 2015). They consist of rhizomes, barks, leaves, fruits, seeds, and other parts of the plant (Tsade, 2016).

Natural food spices such as the Nigella sativa, Ocimum basilicum L. and Ruta chalepensis contain a significant quantity of trace metals (Bhishnurkar et al., 2016). These trace metals in spices and medicinal plants play an important role in living cells. Knowing the composition of trace metals in foods is important because of their essential or toxic in human health (Mubeen et al., 2009). Heavy metals are non-essential for our body even at a low amount. Besides, dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical’s concentration in the environment (Moses and Uwash, 2015). The addition of spices that may be contaminated with trace and heavy metals to food as a habit may result in the accumulation of these metals in human organs (Tsade, 2016). The accumulation of heavy metals can have middle-term and long-term health risks, and strict periodic surveillance of these contaminants is therefore advisable (Mubeen et al., 2009). Micronutrients are important in daily diets, because of their essential nutritious value and possible harmful effects (Moses and Uwash, 2015). Metals like iron (Fe), copper (Cu), zinc (Zn), cobalt (Co) and manganese (Mn) are essential metals since they play an important role in biological systems (Belay et al., 2014). Zinc is important during puberty, pregnancy, and menopause. If large doses of zinc (10–15 times higher than the recommended daily intake) which is 8–15 mg/day are consumed, stomach cramps, nausea, and vomiting may occur (Belay & Abisa, 2015). Besides this, high levels of zinc eating for several months may cause anaemia, damage the pancreas. Consuming a low amount of zinc is a health problem (Belay & Abisa, 2015). Trace metals may enter the food chain from the soil through mineralization by crops or environmental contamination as in the application of agricultural inputs such as pesticides, fertilizers or use of the polluted river for irrigation to water crops (Tsade, 2016).

2. Spices in Ethiopia
In Ethiopia, there are between 6,000 and 7,000 higher plant species, out of about 500 (about 8%) are edible (Belay et al., 2014; Belay & Abisa, 2015). Most Ethiopian spices are cultivated which is grown in home gardens (Belay & Abisa, 2015). In Ethiopia, sweet basil (Ocimum basilicum L.), Black Cumen (Nigella sativa), and Tenadam (Ruta chalepensis) most commonly used as spices (Belay & Abisa, 2015).

3. Black cumin
Black cumin (Nigella sativa L.) belonging to the family Ranunculaceae is an annual flowering plant of which seeds as a spice has been used for decades for both culinary and medicinal purposes. It is also used as a natural remedy for asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, influenza, shows anticarcinogenic and antiulcer activity, antioxidant activities, and antimicrobial activity also acts as carminative, stimulant, and diuretic. The essential oil from the seeds has been found to contain a high concentration of thymoquinone,
thymol and dithymoquinone, which helps in the prevention of inflammation, purge parasites and worms, detoxify, ameliorate amoebic dysentery, shigellosis, abscesses, old tumours, ulcers of the mouth, and rhinitis (Bhishnurkar et al., 2016). According to P.G. (Bhishnurkar et al., 2016), the content of Zn, Cu, Cr, Cd and Pb in Nigella sativa using ICPMS was found 50.76ppm, 22.36ppm, 8.00ppm, 0.04ppm and 24.08ppm, respectively (Bhishnurkar et al., 2016).

4. Sweet basil

_Ocimum basilicum_ L. (sweet basil) belongs to the family Lamiaceae, which includes about 200 species that occur in various botanic varieties and forms. Traditionally, sweet basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhoea, constipation, warts, worms, and kidney malfunctions (Khattak & Khattak, 2011). According to Manzoor Iqbal Khattak and Khattak (2011), the content of Zn, Fe, Cu, Pb and Cd in _Ocimum basilicum_ L. is 27.6 ppm, 242 ppm, 6.72 ppm, 0.26 ppm and 0.25 ppm, respectively.

5. Tenadam

It has blue-green foliage and yellow flowers. It has been in popular herbalism in various countries to treat a variety of ailments. A decoction of the plant has been used in the treatment of paralysis, coughs, and stomachaches. The leaves have been heated and then placed inside the ear to treat earache.

Many analytical methods including Atomic Absorption Spectrometry for trace element determination in plant materials require the digestion of the sample. Because of its sensitivity, specificity, simplicity and precision, Flame Atomic Absorption Spectrometry (FAAS) is the most widely recommended instrument utilized in analytical procedures for trace heavy metal analysis (Belay & Abisa, 2015; Tsade, 2016). The acid digestion method is primarily used to sample preparation and remove organic matrix (Belay & Abisa, 2015; Belay et al., 2014). The main objective of this work was to determine the level of heavy metals, Zn, Fe, Cu, Cd and Pb in commonly consumed spices in Ethiopia using FAAS.

6. Materials and methods

6.1. Description of the study area

The experiment was conducted at three locations of Central Gondar of Ethiopia, which are Takusa, Chuahit, Kolla Diba, and Central Gondar Administrative Zone. The experiment was carried out in Analytical Chemistry Laboratory, Department of Chemistry, College of Natural and Computational Science, University of Gondar.

6.2. Sample collection and processing

| Botanical name   | Family      | English name | Local name | Part used |
|------------------|-------------|--------------|------------|-----------|
| _Nigella sativa_ | Renunculaceae | Black Cumen  | Tikur azmud | Seeds     |
| _Ocimum basilicum_ | Lamiaceae   | Sweet Basil  | Zekakibie  | Flower    |
| _Ruta chalepensis_ | Rutaceae    | Fringed Rue  | Tenadam    | Fruit     |

The sampling technique was random sampling. Sample of three spices ( _Nigella sativa_, _Ocimum basilicum_ and _Ruta chalepensis_) from each sampling site were collected randomly from the mentioned market divided according to the used part of the plant, their scientific, and local names are shown in (Table 1). The three spices sample was washed with tap water and as well with distilled water. Finally, it was rinsed with deionized water and air-dried. The dried whole seed
was powdered and sieved. The powder was collected and stored in clean and dried plastic bottles and then placed in a refrigerator (Mubeen et al., 2009).

6.3. Reagents and chemicals

All reagents and chemicals used in the study were analytical grades. HNO₃ (69–72%) HClO₄ (60–62%) both from (SD Fine Chem Industries Mumbai, India), and H₂O₂ (30%, Schlarau, European Union), and standard stock solutions containing 1000 mg/L, in 2 % HNO₃, of the metals Zn, Fe, Cu, Cd and Pb (BUCK SCIENTIFIC GRAPHIC) were used.

6.4. Digestion of spice sample

Sample digestion was carried out under optimum conditions of Nitric acid-perchloric acid mixture (7:3 v/v), digestion temperature (250 °C) and a total digestion time of 3 hours and 30 minutes. It was applied the procedure, 7 mL concentrated HNO₃ (69–72 %) acid and 3 mL of HClO₄ (60–62 %) (7:3) was added to 0.5 g powdered samples (Bhishnurkar et al., 2016). The mixture was digested under a hot plate by covering the beaker with watch glass until the entire sample was digested and 1–2 mL colorless solution remained. After digestion, the sample was allowed to cool for 10 minutes at room temperature followed by the addition of 10 mL deionized water. The solution was filtered and diluted with deionized water (Belay & Abisa, 2015). In the same way, the blank solution was prepared from 10 mL of reagents (i.e. a mixture of 7 mL of HNO₃ and 3 mL of HClO₄), and then digested the same procedure as the sample (Abdullahi et al., 2008).

6.5. Instruments and apparatus

Polyethylene bags were used for handling and transporting the collected samples, mixer grinder (BRILLIANT 8 x 1002, AN 150 9001:2008 CERTIFIED CAMPANY) pulverizing the dried samples. The digital analytical balance was weighing samples, beaker, used to digest the powder, hot plate (C-MAG HS 4 525, IKA) to digest the dried and powdered samples, fume hood, refrigerator for keeping the digested sample till analysis, pipette, filter paper, 50 mL of volumetric flask. Finally, BUCK SCIENTIFIC 210 VGP flame atomic absorption spectroscopy (FAAS) was analyzed of metals using air-C₃H₂ flame (Khattak & Khattak, 2011; Mubeen et al., 2009).

6.6. Analysis of trace metals in spice sample

Before analysis blank solutions were prepared for calibrating the instrument, it was prepared by digesting the mixture of reagents following the same digestion procedure as the samples and diluted to 50 mL with deionized water (Jinadasa et al., 2010). Measurements were made using the hollow cathode lamps for Zn, Fe, Cu, Cd and Pb at the proper wavelength and the slit width was adjusted using air acetylene flame. Instrumental conditions of experimental setup are shown in Table 2 and the working standards for determination of metals in all spices samples using flame atomic absorption spectrometer shown in Table 3. The concentration of each element in the sample is equal to the quotient of the intercept and the regression coefficient of the calibration curve. Then, each metal content was calculated (using Microsoft excel-2007 and SPSS software version 20) and the results were reported as mean ± standard deviation (Jinadasa et al., 2010). The metal contents of each sample were calculated using the following formula.

| No | Element | Flame Type | Wavelength (nm) | Detection limit (mg/L) | Slit width (nm) |
|----|---------|------------|----------------|------------------------|----------------|
| 1  | Cu      | Acetylene gas | 324.800        | 0.005                  | 0.7            |
| 2  | Fe      | Acetylene gas | 372.000        | 0.050                  | 0.7            |
| 3  | Zn      | Acetylene gas | 213.900        | 0.005                  | 0.7            |
| 4  | Pb      | Acetylene gas | 283.300        | 0.080                  | 0.7            |
| 5  | Cd      | Acetylene gas | 228.900        | 0.010                  | 0.7            |
Metal content (mg/100 g) = \frac{(a-b) \times V}{W}

Where; \( W \) = weight in gm of the sample
\( a \) = concentration in ppm of sample
\( V \) = volume in mL of the extract
\( b \) = concentration in ppm of blank

### 6.7. Evaluation of analytical results

**6.7.1. Precision**

To ensure the precision of the analysis, each sample was digested in triplicate and triplicate reading was carried out (Jinadasa et al., 2010). The precision of the results was evaluated by the relative standard deviation of each metal analysed for each sample. % RSD is calculated as follows:

\[
% \text{ RSD} = \frac{\text{SD}}{\text{X}} \times 100\% \quad (1)
\]

Where: SD = standard deviation of the results
\( X \) = mean concentration value

Using the above formula for all measurements of Zn, Fe, Cu, Cd and Pb % RSD were found in the acceptable range (% RSD < 5) these showed that the result was highly precise.

### Table 3. List of working standards for determination of metals in all spice samples using flame atomic absorption spectrometer

| No | Element | The concentration of standards solution (mg/l) | The correlation coefficient (R) |
|----|---------|-----------------------------------------------|---------------------------------|
| 1  | Zn      | 0.10 0.20 0.40 0.60 0.80                      | 0.9995                          |
| 2  | Cu      | 0.50 1.00 1.50 2.00 2.50                       | 0.9999                          |
| 3  | Fe      | 0.50 1.00 1.50 2.00 2.50                       | 0.9999                          |
| 4  | Cd      | 0.05 0.25 0.50 0.75 1.00                       | 0.9999                          |
| 5  | Pb      | 0.10 0.20 0.40 0.60 0.80                       | 0.9999                          |

### Table 4. Method Instrumental detection limit (IDL), Method detection limit (MDL) = 3Sblank and Quantitation limit (MQL) =10Sblank in mg/L for all metals determined in spices samples (n = 3)

| Metals | MDL(mg/L) | MQL(mg/L) | IDL(mg/L) |
|--------|-----------|-----------|-----------|
| Zn     | 0.1200    | 0.4066    | 0.0050    |
| Cu     | 0.0510    | 0.1690    | 0.0050    |
| Fe     | 0.3970    | 1.3260    | 0.0500    |
| Cd     | 0.0087    | 0.0289    | 0.0100    |
| Pb     | 0.2100    | 0.7000    | 0.0800    |
Table 5. Atomic absorption spectrophotometer results of the metal contents (mean ± SD, N = 3) from Nigella sativa samples (mg/kg)

| Metals     | Zn (mg/100 g) | Cu (mg/100 g) | Fe (mg/100 g) | Cd (mg/100 g) | Pb (mg/100 g) |
|------------|---------------|---------------|---------------|---------------|---------------|
| Kaladiba   | 9.33 ± 0.049  | 2.83 ± 0.021  | 5.55 ± 0.019  | 0.44 ± 0.015  | 2.85 ± 0.0143 |
| Tkusa      | 9.07 ± 0.055  | 2.83 ± 0.043  | 5.55 ± 0.019  | 0.606 ± 0.026 | 5.23 ± 0.082  |
| Chuahit    | 9.59 ± 0.076  | 2.83 ± 0.021  | 1.92 ± 0.019  | 0.80 ± 0.087  | 3.33 ± 0.082  |

6.8. Method detection limit
A method’s detection limit is the smallest amount or concentration of analyte that can be detected with statistical confidence (Jinadasa et al., 2010). To determine the detection limit triplelicate analysis of the four blank samples were performed and the pooled standard deviation of the four reagents blank was calculated. The detection limit was obtained by multiplying the pooled standard deviation of the blank reagent by three.

6.9. Method limits of quantitation (MQL)
Limit of quantification (LOQ) is the smallest concentration or absolute amount of analyte in a sample that can be determined with suitable precision and accuracy. According to the American Chemical Society’s Committee on Environmental Analytical Chemistry, the quantification limit of each element was calculated as ten times the standard deviation of the blank (Jinadasa et al., 2010). If the analyte concentration is below the LOQ value, results are reported as non-detectable. As a result, LOQ is a very important parameter in risk measurement (Jinadasa et al., 2010). The method detection limit, method limit of quantitation and the detection limit of the instrument of each metal have been shown in Table 4.

7. Results and discussion
The Zn, Cu, Fe, Cd, and Pd analyses have been performed on three local spices and the calibration graphics shown in Figure 1a–e.

Heavy metal contents in spices and medicinal plants depend on the age of the plant, climatic factors, plant species, air pollution, mineral composition of soil and other environmental factors (Bhishnurkar et al., 2016). Elemental analysis of Nigella sativa and Ocimum

Table 6. Atomic absorption spectrophotometer results of the metal contents (mean ± SD, N = 3) from Ocimum basilicum samples (mg/kg)

| Metals     | Zn (mg/100 g) | Cu (mg/100 g) | Fe (mg/100 g) | Cd (mg/100 g) | Pb (mg/100 g) |
|------------|---------------|---------------|---------------|---------------|---------------|
| Koladiba   | 8.61 ± 0.067  | 3.58 ± 0.021  | 6.66 ± 0.033  | 0.606 ± 0.0015 | 1.9 ± 0.0082  |
| Tkusa      | 6.92 ± 0.0407 | 2.340.021     | 5.55 ± 0.019  | 0.35 ± 0.0087 | 1.9 ± 0.0082  |
| Chuahit    | 7.84 ± 0.085  | 3.086 ± 0.021 | 4.44 ± 0.019  | 0.44 ± 0.0017 | 3.33 ± 0.0082 |

Table 7. Atomic absorption spectrophotometer results of the metal contents (mean ± SD, N = 3) from Ruta chalepensis samples (mg/kg)

| Metals     | Zn (mg/100 g) | Cu (mg/100 g) | Fe (mg/100 g) | Cd (mg/100 g) | Pb (mg/100 g) |
|------------|---------------|---------------|---------------|---------------|---------------|
| Koladiba   | 7.55 ± 0.030  | 6.17 ± 0.021  | 5.55 ± 0.019  | 0.45 ± 0.0026 | 2.85 ± 0.0014 |
| Tkusa      | 8.66 ± 0.05   | 9.01 ± 0.021  | 6.66 ± 0.033  | 0.45 ± 0.0015 | 1.9 ± 0.0082  |
| Chuahit    | 9.38 ± 0.0705 | 9.38 ± 0.021  | 4.44 ± 0.019  | 0.707 ± 0.0017| 1.9 ± 0.0082  |
basilicun seeds has revealed the presence of metals of Zn > Fe > Cu > Pb > Cd shown in Tables 5 and 6 respectively. Whereas in Ruta chalepensis seed sample showed the order of Zn > Cu > Fe > Pb > Cd Table 7. The metal concentration of Nigella sativa, Ocimum basilicun, and Ruta chalepensis seeds by FAAS.

Zinc is an essential human nutrient; it acts as a catalyst for over 300 enzymes and is found in all tissues. It plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division (Bhishnurkar et al., 2016). According to the national institute of health, the RDA of zinc for an adult is 11 mg/day for males and 8 mg/day for females. In this study, the Zn content of Nigella sativa, Ocimum basilicun and Ruta chalepensis were between 9.59 mg/100 g and 7.55 mg/100 g. Therefore those spices are a good source of Zn.

Even a small amount of toxic metals can cause severe damages to human and other living organisms, thus investigations and control of heavy metals are important. Also, the monitoring of metals in these spices is therapeutically important. In this work, Nigella sativa, Ocimum basilicun and Ruta chalepensis are good sources of essential metals Zn, Cu, Fe and the number of toxic metals Cd and Pb are below the
permissible limit as compared to the value reported in WHO. The standard level of Pb and Cd in some spices recommended by FAO/WHO is 0.30 mg kg⁻¹ and 0.20 mg kg⁻¹ respectively (Al-Eed et al., 2010).

8. Conclusion

Flame atomic absorption spectroscopy was used to determine the concentration of heavy metals (Zn, Cu, Fe; toxic metals Cd and Pb) in *Nigella sativa*, *Ocimum basilicum* and *Ruta chalepensis* varieties with wet digestion method under optimum conditions of nitric acid- perchloric acid mixture used as a reagent. This study confirms that *Nigella sativa*, *Ocimum basilicum* and *Ruta chalepensis* were found to contain essential metals Zn, Cu, Fe; toxic metals Cd and Pb were found below the permissible limit as compared to the value reported in WHO. Therefore, a non-carcinogenic health risk to the consumer associated with the consumption of this spices preparation marketed in study areas.

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The authors declare that there are no conflicts of interest.

Ethical declarations

No need for an ethical declaration

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