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Determination of selected heavy metals in leaves of the medicinal plants *Calpurnia aurea* and *Zehneria scabra* from East Gojjam, Ethiopia, and their health risk assessment

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ABSTRACT

This study investigates the levels of selected heavy metals (Zn, Pb, Cd, and Cr) in the leaves of *C. aurea* and *Z. scabra.* Flame atomic absorption spectrophotometry (FAAS) was used for the determination of these metals. Wet digestion of the *C. aurea* and *Z. scabra* required 4:1 HNO_3/HCl (v/v) for 1:45 h, with a temperature of 210 °C and 3:1 HNO_3/HCl (v/v) for 1:30 h, with a temperature of 240 °C, respectively, for complete digestion of 0.5 g of both medicinal plant leaves using the Kjeldahl apparatus. The experimental results revealed that the metals studied found in the plants were determined as follows, in mg/kg: Zn (0.5727, 0.4547); Pb (0.2394, 0.2334) and Cd (0.0365, 0.0468) in *C. aurea* and *Z. scabra*, respectively, while Cr was not detected in both plant samples. The recovery percentages for *C. aurea* and *Z. scabra* samples ranged from 88.2-95.8% and 90.0-98.9%, with LOD values of 0.0087-0.1476 and 0.0174-0.1176, respectively. The levels of these metals were found to be below the allowable limits of the World Health Organization. The plant *C. aurea* was found to contain higher concentrations of Zn and Pb compared to *Z. scabra*, which had a slightly higher Cd level. The hazard quotient and hazard index values of Zn, Pb, and Cd in both medicinal plants were lower than one, which may ensure that these plant spices do not pose substantial health risks to consumers.

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1. Introduction

Medicinal plants have played a crucial role in human health care providing natural cures and therapeutic substances for thousands of years [1]. These plants have special bioactive substances that can help heal, reduce symptoms, and stop illnesses. The use of medicinal plants is widespread across nations and customs, adding to the diverse range of herbal medicines used around the world [2]. Approximately 80% of Africans receive their primary medical care from medicinal plants [3]. A significant proportion of people around the world choose medicinal plants, which constitute alternative medicine for contemporary synthetic pharmaceuticals [4]. Medicinal plants are gaining recognition within modern medicine [3]. Pharmacology often derives new drugs from plant compounds and acknowledges their potential in treating various diseases. Several pharmaceutical drugs are based on or are inspired by the principles of traditional herbal medicine. For example, *Calpurnia aurea* and *Zehneria scabra* are significant medicinal plants known for their therapeutic benefits. Their incorporation into traditional and contemporary medicine underscores the continuous interest in natural health remedies.

The name *C. aurea* translates to "golden" in Latin, while *sylvatica* means "growing among trees." This species was first described in 1789, based on a plant found at the Royal Botanic Gardens, Kew, which is believed to have been introduced from Ethiopia in 1777 [5]. *C. aurea* (Digitta or Zigitta in Amharic) is a flowering plant belonging to the Fabaceae (legume) family [6,7]. It is typically a shrub or small tree characterized by its bright yellow flowers and compound leaves. The plant can grow in various habitats, often in savannahs and open forests. Native to parts of Africa, the plant is particularly found in regions such as South Africa, Botswana, Ethiopia, and Namibia [8,9]. Various parts of the plant, including the leaves and roots, are used in folk medicine to treat ailments such as respiratory problems, digestive problems, and skin conditions [9-11]. Some studies suggest that *C. aurea* may have antimicrobial effects, making them useful in treating infections [12].

Z. scabra was first described by Endlicher based on collections made by Bauer on Norfolk Island in 1804–1805 [13].

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Z. scabra (Hreg-resa in Amharic), commonly known as the rough cucumber, is a climbing or trailing herbaceous plant in the *Cucurbitaceae* family [14]. It has lobed leaves and produces small yellowish flowers, followed by cucumber-like fruits. This species is widely distributed across tropical and subtropical regions of Africa. *Z. scabra* contains bioactive compounds that contribute to its medicinal properties [15,16]. Traditionally, *Z. scabra* has been used to alleviate gastrointestinal issues such as diarrhea and stomach cramps. The plant is also noted for its potential anti-inflammatory properties [14,16].

C. aurea and Z. scabra are prominently utilized in cultural and traditional therapeutic practices. Their significance in these applications reflects the deep-rooted connection between natural remedies and cultural heritage. These medicinal plants contain a range of bioactive compounds such as flavonoids, saponins, alkaloids, tannins, glycosides, essential oils, vitamins, and minerals [9]. These plants reflect the integration of local biodiversity into traditional health practices, showcasing the cultural significance of herbal remedies in Ethiopian society. Although these plants offer numerous health benefits, there are significant health risks, particularly concerning heavy metal poisoning associated with their extraction and cultivation [1,2]. There is limited information on the level of heavy metals and consumers may be at risk from higher concentrations of the various metal constituents [2]. Since the symptoms of their toxicities are generally not severe, the risks may worsen in most circumstances. Therefore, stricter regulations and quality control over medicinal plants are required as the demand for natural medicines increases. When herbal products are not properly tested and labeled, consumers can unintentionally be exposed to dangerous levels of heavy metals. Therefore, this study aimed to assess the levels of heavy metals and assess the possible health risks associated with the consumption of widely cultivated spices from *C. aurea* and *Z. scabra* to ensure safety and efficacy in herbal medicine.

2. Experimental

2.1. Sampling sites and sample collection

The leaf samples of the *C. aurea* and *Z. scabra* plants were collected from Debre Work area, located in the east Gojjam in the district of Enarj Enawga of the Amhara Regional state, Ethiopia. A total of 100 g of representative fresh leaves from each plant sample were collected in polyethylene bags from two locations of the sampling sites. The specific sampling location found in the area of Debre Work town, which is 195 km south east of Bahir Dar; the capital city of Amhara Regional state, and 291 km north of Addis Ababa, the capital city of Ethiopia. Its geographical coordinates are 10°49'59.99" N latitude and 38°04'60.00" E longitude, with elevation of 2592 m above sea level. The average temperature of the area is 22.5 °C, with a measured annual rainfall of 1228 mm. Each plant sample was packed in separate clean plastic bags, labeled, and transported to the Analytical Chemistry Laboratory of the Addis Ababa University (AAU) for subsequent analyses.

2.2. Chemicals and reagents

The chemicals and reagents used in this study were analytical grade reagents. The digestion of *C. aurea* and *Z. scabra* plant samples and blank solutions were carried out using 69.72% HNO_3 and 70% $HClO_4$ (Research-Lab Fine Chem. Industries, India). Distilled deionized water was used throughout the experimental work for sample preparation, dilution and rinsing (cleaning) of the apparatus prior to and during analysis. Standard solutions of the selected heavy metal ions, i.e., Pb, Cd, Zn, and Cr were obtained from Anapure Kriat, Daejeon, Korea; were freshly prepared from stock standard solutions of 1000 mg/L; from which intermediate standard solution of 100 mg/L were prepared for a series of analytical works. Diluted solutions of appropriate concentrations were prepared for calibration and determination of the amounts of metals in the samples and for spiking experiments.

2.3. Apparatus and instruments

All the apparatus and equipment used to carry out the acid digestion were carefully cleaned and rinsed in order to free them from the possible contamination and dusts. Volumetric flasks and ceramic mortar and pestle were regularly cleaned using a concentrated solution of HNO_3 followed by rinsing with distilled and deionized water before use. All other glassware and common apparatus employed in this study were first washed with detergents and then soaked in 10% HNO_3 for 24 h. They were then rinsed with distilled deionized water three times and finally kept in an oven for further drying at 70-80 °C.

2.4. Preparation of metals ion solutions

Standard solutions of 1000 mg/L of the selected heavy metals (Pb, Cd, Zn, and Cr) were prepared from their respective nitrate salts. To this end, appropriate amounts of metallic salts were dissolved in distilled deionized water and diluted to a final volume of 1000 mL with additional deionized water. Subsequently, these stock solutions were diluted serially with distilled deionized water to obtain the test or working solutions at the desired concentrations. Working solutions were prepared daily and stored in a refrigerator at 4 °C. The concentration measurement of the target metal ions was performed using a flame atomic absorption spectrophotometer (FAAS) (Model 700P Zeenit, Germany), using hollow cathode lamps and air acetylene flame. Calibration graphs were generated by plotting absorbance of the measured ions against their corresponding concentrations throughout the laboratory works.

2.5. Sample preparation and acid digestion

The *C. aurea and Z. scabra* plant leaves were cut down into pieces using a stainless-steel Teflon (PTFE) knife and then stored into polyethylene bags. The leaves were then transferred to a crucible that was kept in the air-circulating oven (Digitheat, JP Selecta, Spain), for 72 h; to ensure subsequent drying of the samples at 75 °C for 48. The dried leaf samples were then ground and homogenized, into a fine powder, using ceramic mortar and pestle, which were separately sieved to obtain a fine powder that can pass through a 0.5 mm sieve. Finely powdered samples were ultimately stored in clean polyethylene plastic bags and kept in a desiccator until acid digestion.

For sample preparation of the processed plant samples by acid digestion, each homogenized leaf sample of *C. aurea* and *Z. scabra*, 0.5 g powder, was accurately weighed using a digital analytical balance (SCIENTECH, ZSA, 120; with ± 0.0001 g precision). Then, the measured powder was quantitatively transferred to 250 mL round bottom flasks. A 5 mL of a 4:1 (v/v) mixture of HNO_3 (69.72%) and $HClO_4$ (70%) was added to C. *aurea*, while a 4 mL, *i.e.*, 3:1, mixture of the same acids was used for *Z. scabra*. The mixtures were digested on a Kjeldahl heating apparatus (Gallenhamp, England), with the temperature set at 210 °C for 1 h and 45 min for the first sample and at 240 °C for 1 h and 30 min for the latter [17-20]. After digestion, the mixtures were allowed to cool for 20 min with the condenser in place, followed by additional 10 min cooling time after the condenser was removed. To the cooled solutions, 10 mL distilled deionized water was further added to complete the dissolution of any precipitate.

Filtrations of the digested mixtures were achieved in the filtration funnel (Kenutuf, England), through a 110 mm diameter filter paper (Ltd Maid-Stone, England).

Table 1. Optimization of the volume ratio of the reagent for digestion of the 0.5 g of *C. aurea* leaf sample.

Trial	HNO_3:$HClO_4$ ratio (mL)	Volume (mL)	Temperature (°C)	Time (h)	Observations
1	1:1	2	300	3:00	Yellow solution
2	2:1	3	300	3:00	Yellowish solution
3	3:1	4	300	3:00	Pale yellow solution
4	2:2	4	300	3:00	Colorless but not clear
5	4:1 *	5	300	3:00	Clear and colorless solution
6	3:2	5	300	3:00	Clear solution

* Indicates optimum volume ratio of the reagents for digestion.

Table 2. Optimization of the digestion temperature for the 0.5 g of *C. aurea* leaf sample.

Trial	Optimized reagent ratio (mL)	Volume (mL)	Temperature (°C)	Time (h)	Observations
1	4:1	5	90	3:00	Deep yellow solution
2	4:1	5	120	3:00	Yellow solution
3	4:1	5	150	3:00	Yellow solution
4	4:1	5	180	3:00	Dim yellow solution
5	4:1	5	210 *	3:00	Clear and colorless solution
6	4:1	5	240	3:00	Clear and colorless solution

* Indicates optimum digestion temperature.

Table 3. Optimization of the digestion time for the 0.5 g of *C. aurea* leaf sample.

Trial	Optimized reagent ratio (mL)	Volume (mL)	Optimized temperature (°C)	Time (h)	Observations
1	4:1	5	210	1:00	Yellow solution
2	4:1	5	210	1:10	Yellowish solution
3	4:1	5	210	1:20	Blurred yellow solution
4	4:1	5	210	1:30	Colorless but not clear
5	4:1	5	210	1:45 *	Clear and colorless solution
6	4:1	5	210	2:00	Clear and colorless solution

* Indicates optimum digestion time.

Table 4. Optimization of the volume ratio of the reagent for digestion of the 0.5 g of *Z. scabra* leaf sample.

Trial	HNO_3:$HClO_4$ ratio (mL)	Volume (mL)	Temperature (°C)	Time (h)	Observations
1	1:1	2	270	2:30	Yellow solution
2	2:1	3	270	2:30	Yellowish solution
3	3:1 *	4	270	2:30	Clear and colorless solution
4	2:2	5	270	2:30	Very pale-yellow solution
5	4:1	5	270	2:30	Colorless but not clear
6	3:2	5	270	2:30	Colorless but not clear

* Indicates optimum volume ratio of the reagents for digestion.

Table 5. Optimization of the digestion temperature for the 0.5 g of *Z. scabra* leaf sample.

Trial	Optimized reagent ratio (mL)	Volume (mL)	Temperature (°C)	Time (h)	Observations
1	3:1	4	120	2:30	Yellowish solution
2	3:1	4	150	2:30	Yellowish solution
3	3:1	4	180	2:30	Very pale-yellow solution
4	3:1	4	210	2:30	Colorless but not clear
5	3:1	4	240 *	2:30	Clear and colorless solution *
6	3:1	4	270	2:30	Clear and colorless solution

* Indicates optimum digestion temperature.

Table 6. Optimization of the digestion time for 0.5 g of *Z. scabra* leaf sample.

Trial	Optimized reagent ratio (mL)	Volume (mL)	Temperature (°C)	Time (h)	Observations
1	3:1	4	240	1:00	Yellowish solution
2	3:1	4	240	1:10	Light yellow solution
3	3:1	4	240	1:20	Colorless but not clear
4	3:1	4	240	1:30 *	Clear and colorless solution
5	3:1	4	240	1:45	Clear and colorless solution
6	3:1	4	240	2:00	Clear and colorless solution

* Indicates optimum digestion time.

The filter paper was then rinsed with 5 mL distilled deionized water, which was also used to dilute to the 25 mL mark. The digestion process was carried out in triplicate for both plant samples. In addition, three blank reagent solutions were also prepared using the same digestion procedure. All digested samples and blanks were kept for heavy-metal analysis using flame atomic absorption spectrophotometry (FAAS). For recovery studies, known concentration of the standard solutions were spiked using cleaned micropipettes (Shanghai, China).

2.6. Optimization of acid digestion of C. aurea and Z. scabra leaf samples

The optimization of the sample preparation procedures, in order to establish appropriate digestion conditions, for each plant sample, *C. aurea* and *Z. scabra*, was described as follows: The purpose of these experiments was to fix the minimum volume of the acids and to reduce the time required to obtain clear solutions of the extracts. In these experiments, concentrated solutions of HNO_3 and $HClO_4$ were used with careful adjustments of the parameters such as acid volume ratio, temperature and time required to achieve complete digestion [21]. The Kjeldahl heating apparatus was used to digest the plant leaves, where organic components decompose by releasing gases, while non-volatile metallic elements remain in solution [22]. The digestion was considered complete when the resulting solution appeared clear and colorless. The optimization conditions established for *C. aurea* and *Z. scabra* are provided in Tables 1-6.

2.7. Analysis and quality assurance

A series of working standard solutions of the studied metal ions were prepared from a 10 mg/L intermediate standards; obtained from diluted stock solutions of 1000 mg/L for each metal ion, *viz.*; chromium (Cr), lead (Pb), cadmium (Cd) and zinc (Zn). Analysis was conducted using the FAAS instrument. An external calibration curve was used after optimizing parameters for maximum signal intensity. Three replicate measurements were performed for each sample, using hollow cathode lamps under recommended conditions [23]. Concentrations were determined at specific wavelengths: Cr (357 nm), Pb (283 nm), Cd (228 nm) and Zn (213 nm). Under the optimized conditions, four standard concentrations; 0.25, 0.50, 0.75 and 1.00 mg/L were measured within the linear dynamic range and calibration curves were plotted starting from the limits of detection. Replicate analyses and spike recovery measurements were used to further validate the method's accuracy [24,25].

2.7.1. Calculation of the limit of detection

The limit of detection (LOD) is the lowest concentration level that can be determined to be statistically different from the analyte blank or is the minimum concentration that can be detected by the analytical method with a given confidence limit [26]. According to the United States Environmental Protection Agency (USEPA), LOD is the minimum concentration of a substance that can be measured and reported with 99% confidence level that the analytical concentration is greater than zero. The generally accepted and common definition of LOD is the concentration that gives a signal three times the standard deviation of the blank, $S_{blank,}$ or background signal, as shown in Equation 1 [26].

$$\mathrm{LOD} = 3 \times S_{\mathrm{blank}}, n = 6 \quad (1)$$

2.7.2. Recovery study (R)

Recovery analysis of the analytical method is performed using the results obtained from the nonspiked and spiked samples, usually in triplicate, and a known amount of the analyte added to the sample solution. Then, in order to obtain the experimental result, the recovered quantity/percent of the added amount is estimated according to the following relation; Equation 2:

$$\%\mathrm{Recovery} = \frac{\text{Conc.in spiked sample} - \text{Conc.in non-spiked sample}}{\text{Amount added}} \times 100 \quad (2)$$

2.8. Health risk assessment

Health risk assessment (HRA) is a methodical procedure used to assess the possible health risks associated with a person's surroundings, way of life, and demographics. It seeks to recognize, evaluate, and control risks to improve health outcomes and guide clinical and public health decision making [27]. The health risks of heavy metals through consumption of *C. aurea* and *Z. scabra* were evaluated by estimated dietary exposure, target hazard quotient, hazard index, and metal pollution load index.

2.8.1. Estimated daily intake (EDI)

The estimated daily intake (EDI) of the metals assessed in this study, by the adult population, was calculated based on the mean concentration of each metal in the medicinal plants and their corresponding consumption rates. The most common approach to evaluate health risks associated with metals in food involves calculating anticipated dietary intake values and comparing them to establish dietary standards and acceptable upper intake levels. EDI is commonly calculated from the average daily intake of herbal preparations; in mg/kg per day in food of the body weight. EDI estimates for different population groups are expressed according to Equation 3 [27-30].

$$\text{Estimated daily intake (EDI, mg/kg body weight)} = \frac{\text{Conc. (mg/kg)} \times \text{Conc. (0.1 kg/day)}}{\text{Body weight (kg)}} \quad (3)$$

Where the average body weight in kg/person of an adult consumer is 65 kg [30].

2.8.2. Health risk index (HRI)

The health risk index (HRI) is a measure used to evaluate the potential health risks associated with exposure to contaminants such as heavy metals in food, soil, or water. The specific health risks related to each heavy metal tested in the medicinal plant, which can accumulate, are represented by the health risk index (HRI), given by Equation 4:

$$\text{Health risk index (HRI)} = \sum \text{Target hazard quotient (THQ)} \quad (4)$$

where THQ represents the target hazard quotient. An HRI value greater than 1 suggests that exposure levels exceed safe thresholds, indicating a higher risk of adverse health effects. In contrast, an HRI value below 1 implies that exposure to these leaves may be unlikely to cause health risks to consumers.

2.8.3. The target hazard quotient (THQ)

The THQ is a risk assessment metric used to evaluate the potential non-cancer health risks associated with exposure to environmental contaminants, particularly heavy metals. It is estimated from heavy metal-contaminated foods consumed and is obtained from the relationship between exposure and the reference oral dose, as defined in Equation 5. If the ratio is less than 1, the population would not be at risk; if it is equal to or greater than 1, the population will be at risk of health problems [28].

$$\text{Target hazard quotient (THQ)} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C}}{\mathrm{RfD} \times \mathrm{BW} \times \mathrm{AT}} \times 10^3 \quad (5)$$

where EF stands for exposure frequency (365 days annually) and ED is exposure duration, equivalent to the average lifetime where according to the World Health Organization life expectancy at birth [31]; the average life time of Ethiopian men is 64 years and women is 67 [31]; FIR is the food intake rate, 100 and 50 g/day for an adult and children, respectively; C stands for the metal concentration (mg/kg) present in plants; RfD stands for the reference oral dose, an estimate of the daily exposure of a contaminant to which the human population may be continually exposed for a lifetime without appreciable risk of harmful effects; mg/kg per day. The RfD values for Cu and Zn are 0.04 and 0.30 mg/kg per day, respectively [30], and the RfD values for Pb, Cd and Cr are 0.004, 0.001 and 0.003 mg/kg per day, respectively [32]. BW represents the body weight of an adult (65 kg) and a child (12 kg, less than 3 years) consumer [33], and AT is the average exposure time (365 days/year x the number of years of exposure).

3. Results and discussion

The calibration data information for the four heavy metals; *viz.*, Cr, Pb, Cd, and Zn, is summarized in Table 7.

Table 7. Calibration parameters for the FAAS determination of selected metals.

Metals	Stock solution (mg/L)	Intermediate concentration (mg/L)	Working standard concentrations (mg/L)	R^2	Calibration equation
Cr	1000	10	0.25, 0.50, 0.75, 1.00	0.9963	y=0.0860x - 0.0006
Pb	1000	10	0.25, 0.50, 0.75, 1.00	0.9993	y=0.0327x + 6.9×10^{-3}
Cd	1000	10	0.25, 0.50, 0.75, 1.00	0.9959	y=0.2149x + 3.6×10^{-3}
Zn	1000	10	0.25, 0.50, 0.75, 1.00	0.9971	y=0.5459x + 8.0×10^{-3}

Table 8. Limit of detection (LOD) for the determination of metal ions in *C. aurea* and *Z. scabra* leaf samples *.

***C. aurea* leaf samples**				***Z. scabra* leaf samples**		
Metals	**Mean (mg/L)**	**SD**	**LOD (mg/L)**	**Mean (mg/L)**	**SD**	**LOD (mg/L)**
Zn	0.3109	0.0492	0.1476	0.2408	0.0392	0.1176
Pb	0.1483	0.0117	0.0351	0.1125	0.0098	0.0294
Cd	0.0177	0.0029	0.0087	0.0227	0.0058	0.0174
Cr	ND	-	-	ND	-	-

* SD stands for standard deviation and ND stands for not detected.

Table 9. Recovery analysis results for *C. aurea* and *Z. sacbra* leaf samples *.

Metals	**Concentration in *C. aurea* leaf**					**Concentration (mg/kg) in *Z. sacbra* leaf**				
	A (mg/kg)	**B (mg/kg)**	**C (mg/kg)**	**D±SD (mg/kg)**	**%R**	**A (mg/kg)**	**B (mg/kg)**	**C (mg/kg)**	**D±SD (mg/kg)**	**% R**
Zn	0.5727±0.0268	0.1450	0.7052±0.0397	0.1325±0.0128	91.4	0.4547±0.0365	0.0410	0.4916±0.0305	0.0369±0.0037	90.0
Pb	0.2394±0.0098	0.0958	0.3312±0.0183	0.0918±0.0090	95.8	0.2334±0.0098	0.0169	0.2501±0.0110	0.0167±0.0015	98.9
Cd	0.0365±0.0020	0.0153	0.0500±0.0034	0.0135±0.0014	88.2	0.0468±0.0012	0.0023	0.0491±0.0016	0.0022±0.0003	95.7
Cr	ND	0.0147	0.0142±0.0018	0.0142±0.0017	96.6	ND	0.0134	0.0128±0.0023	0.0128±0.0014	95.5

* A = Unspiked sample, B = Amount spiked, C = Sum of spiked and unspiked concentrations, D = Recovered amount, %R = Percent recovery, and ND = Not detected.

Table 10. Average concentrations (mean±SD) of heavy metals in *C. aurea* and *Z. scabra* leaf samples *.

Metals	***C. aurea* leaf**		***Z. scabra* leaf**	
	x̄±SD (mg/kg)	**RSD**	**x̄±SD (mg/kg)**	**RSD**
Zn	0.5727±0.0269	4.67	0.4547±0.0365	8.03
Pb	0.2394±0.0098	4.09	0.2334±0.0098	4.20
Cd	0.0365±0.0020	5.48	0.0468±0.0012	2.56
Cr	ND	-	ND	-

* x̄ = Mean; RSD = relative standard deviation; ND = Not detected.

Each metal was prepared from a stock solution of 1000 mg/L, diluted to an intermediate concentration of 10 mg/L, and further diluted to obtain standard concentrations of 0.25, 0.5, 0.75 and 1.0 mg/L. All metals exhibit high correlation coefficients (R^2) ranging from 0.9959 to 0.9993, indicating a strong linear relationship between concentration and absorbance for each metal. This suggests that the calibration curves are reliable for quantifying the target metals. Average trace element concentrations for both *C. aurea* and *Z. scabra* are reported in mg/L (mean±standard deviation).

3.1. Limit of detection of the studied metals

The limit of detection, LOD, was estimated from six analytical blanks; both *C. aurea* (digested in a mixture of 4 mL of HNO_3 and 1 mL of $HClO_4$) and *Z. scabra* leaf samples (in a mixture of 3 mL of HNO_3 and 1 mL of $HClO_4$) obtained from the optimized procedure. Triplicate analyzes of six blank samples for all elements were performed and the pooled standard deviations of the six blank reagents were calculated. The detection limits of the metals studied, Table 8, were obtained by multiplying the pooled standard deviation of the reagent blank by three using Equation 1.

3.2. Recovery studies

The recovery value of the metals studied in the spiked leaf samples was found to vary from 88.2% to 95.8% for *C. aurea* and 90.0% to 98.9% for *Z. scabra*, respectively; Equation 2. Determination of the recovery was based on the average values of three measurements of the spiked and un-spiked samples. In addition, it was observed that the entire recovery range, for both leaves of the plants, was found to vary between 88.2 and 98.9%, Table 9. The results obtained revealed that the optimized digestion procedure effectively removes metal fractions associated with organic matter, confirming the accuracy and precision of the method [25].

3.3. Mean concentration of selected heavy metals in C. aurea and Z. scabra leaf samples

The mean concentrations of the selected heavy metals (Zn, Cd, Cr, and Pb) in the leaf samples of the *C. aurea* and *Z. scabra* plants are presented in Table 10. The mean concentrations of Zn and Pb were found to be higher in the medicinal plant of *C. aurea*, while the mean concentration of Cd was found to be relatively higher in the medicinal plant of *Z. scabra*. Both plant species have low variability in their measurements for Pb and Cd. Cr was not detectable in both medicinal plants. These results might indicate acceptable assessments of metal accumulation in the plant species.

The standard deviation (SD) measures the variation in metal concentrations across samples. A low SD indicates high precision in flame atomic absorption spectroscopy (FAAS), while a high SD suggests greater variability, which may arise from inconsistencies in sample preparation or instrument sensitivity [25]. Both SD and relative standard deviations (RSD) are critical parameters to evaluate measurement precision. The lower RSD values for Pb in *C. aurea* and for Pb and Cd in *Z. scabra* may indicate reliable FAAS results. The higher RSD for Zn in *Z. scabra* highlights potential measurement challenges, including lower precision and increased variability.

3.4. Concentration levels of Zn in leaf samples from C. aurea and Z. scabra

The mean concentration of Zn found in the leaf samples of *C. aurea* and *Z. scabra* were 0.5727±0.0269 mg/kg and 0.4547±0.0365 mg/kg, respectively. The amount of Zn determined in both medicinal plants was found to be below the consumption level (50 mg/kg) for medicinal or herbal plants [34], indicating that the trace level of Zn, in both plant samples, may not cause adverse risk to consumers. It is also known that Zn is essential for various metabolic processes in plants, functioning as a component of several enzymes such as dehydrogenases, proteinases, peptidases, and phosphor-

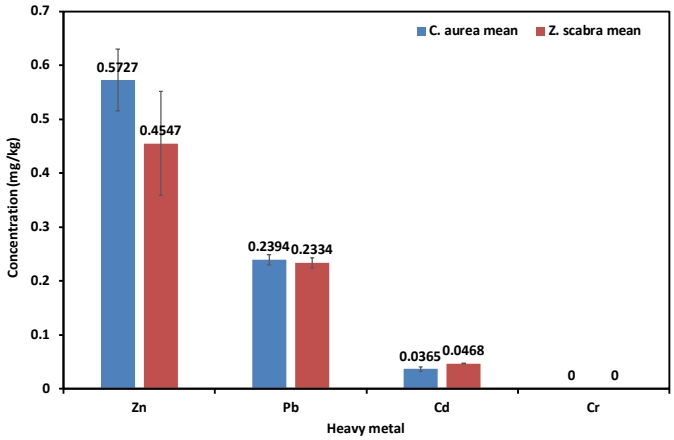

Figure 1. Levels of mean concentration (mg/kg) in the sample of *C. aurea* and *Z. scabra* leaves.

hydrolases [35,36]. However, the appearance of excessive Zn may cause toxicity, manifesting itself as symptoms such as chlorosis in young leaves, browning of coralloid roots, and significant inhibition of plant growth [37].

3.5. Concentration levels of Pb in leaf samples from C. aurea and Z. scabra

The determined concentrations of Pb were found to be 0.2394±0.0098 mg/kg in *C. aurea* and 0.2334±0.0098 mg/kg in *Z. scabra* medicinal plants, respectively. The acceptable concentration limit for Pb, both in herbal and medicinal plants, is 2 mg/kg [38,39]. The amounts of Pb found in the plant leaves were below the allowed levels, indicating that neither plant is said to be adversely contaminated by Pb [40]. Pb is a known toxic element with no safe exposure level, making it essential to minimize all exposure to Pb in our environment. Pb can often accumulate significantly in plants without showing visible symptoms that affect the appearance or yield of plants, and therefore can cause a harmful effect to plants [41]. The human body can absorb lead through inhalation, ingestion, or dermal contact, with ingestion typically occurring through the consumption of contaminated vegetables. However, plants normally absorb Pb from soils through their roots [42].

3.6. Concentration levels of Cd in leaf samples from C. aurea and Z. scabra

In this study, the mean Cd concentrations were found to be 0.0365±0.0020 mg/kg for *C. aurea* and 0.0468±0.0012 mg/kg for *Z. scabra*; with *Z. scabra*, showing a higher Cd concentration compared to *C. aurea*. Although Cd can be toxic and pose serious health risks to humans, both concentrations found in the studied plant samples were below the recommended limit of the World Health Organization of 0.2 mg/kg [38]. This may confirm that there is no Cd contamination in either plant sample that may be causing health risks. It is important to note that both Pb and Cd are not essential for human health or plant growth, and they can lead to various adverse effects even at low exposure levels. Over time, Cd can accumulate in the body, potentially resulting in chronic health problems such as osteoporosis and cancer [43].

3.7. Concentration levels of Cr in leaf samples from C. aurea and Z. scabra

The maximum permissible limit for Cr in herbal or medicinal plants is 1.30 mg/kg [38,44]. In this study, Cr was not detected in the leaf samples of either *C. aurea* or *Z. scabra*. This absence may be due to the occurrence of Cr at low concentrations in the underlying soil, as well as the type, nature and chemical form of the soil. The fact that Cr levels are below the detection limit indicates and reassuring that these plants do not accumulate significant amounts of Cr. Cr exists in various oxidation states, with trivalent chromium (Cr(III)) being an essential nutrient in small amounts, while hexavalent chromium (Cr(VI)) is highly toxic. The undetectable levels of Cr in this study may indicate a lower incidence of causing toxicity, especially with respect to the potential presence of Cr(VI).

The bar graph shown in Figure 1 shows the concentrations of heavy metals Zn, Pb, Cd, and Cr in the two-plant species: *C. aurea* (blue) and *Z. scabra* (red), measured in mg/kg. The highest concentration is for Zn, with *Z. scabra* at 0.5727 mg/kg and *C. aurea* at 0.4547 mg/kg, indicating that *Z. scabra* has a greater bioaccumulation capacity. Pb levels are lower, with *C. aurea* at 0.2394 mg/kg and *Z. scabra* at 0.2334 mg/kg, indicating similar accumulation. Cd concentrations are low, at 0.0365 mg/kg for *C. aurea* and 0.0468 mg/kg for *Z. scabra*. Cr is not detected in either plant species, suggesting that it may occur at the trace level in the environment or is effectively avoided by both species. The heavy metal concentrations in both plants are within safe limits, with no significant contamination detected indicating that the levels of heavy metals Zn, Pb, Cd, and Cr found in leaf samples from both *C. aurea* and *Z. scabra* are below established safety thresholds [45-47]. It has been observed that concentrations of all measured metals were below the World Health Organization's maximum allowable limits for medicinal plants: Zn (50 mg/kg), Cd (0.2 mg/kg), and Pb (2 mg/kg) [48].

3.8. Comparison of the metal content in the plants leaves with other leaves of medicinal plants

The medicinal plants considered in this study *(C. aurea* and *Z. scabra)* contained relatively low concentrations of heavy metals studied, making them safer options for medicinal uses compared to other plants with which comparisons were made, Table 11.

The levels of heavy metals in the plants of *C. aurea* and *Z. scabra* were compared with other medicinal plants reported in Egypt [49] and Thailand [50], revealing that the findings align well with the existing literature, except for the levels of Zn and Pb in the leaves of *Marjorana hortensis* from Egypt. In this study, the mean Zn concentrations in *C. aurea* and *Z. scabra* were lower than those reported for other medicinal plants in both countries, as shown in Table 11. Similarly, the mean Pb concentrations were also lower than those found in the literature.

Table 11. Comparison of the heavy metal concentration in *C. aurea* and *Z. scabra* leaves with those reported for other medicinal plants.

Medicinal plants	Origin	Part used	Heavy metal concentration				Reference
			Zn (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	
Pelargonium graveolens L.	Egypt	Leaves	12.1	-	-	-	[49]
Marjorana hortensis L.	Egypt	Leaves	10.59	14.4	2.05	2.15	[49]
Gynostemma pentaphyllum	Thailand	Leaves	25.43-61.95	0.361-64.40	0.021-4.772	0.434-12.42	[50]
Camellia sinensis	Thailand	Leaves	10.13-55.40	0.060-53.89	0.002-0.100	0.205-10.54	[50]
Morus alba	Thailand	Leaves	19.16-34.4	0.118-1.185	0.001-0.022	0.250-1.419	[50]
Hibiscus schizopetalus	Nigeria	Leaves	19.67±1.19	1.71±0.08	0.51±0.69	1.96±0.88	[51]
Ficus thonnigi	Nigeria	Leaves	15.93±0.83	9.01±1.84	0.001	0.43±0.14	[51]
Calpurnia aurea	Ethiopia	Leaves	0.5727	0.2394	0.0365	ND	This study
Zehneria scabra	Ethiopia	Leaves	0.4547	0.2334	0.0468	ND	This study

Table 12. Estimated daily intake (EDI, mg/kg/day), target hazard quotient (THQ), and hazard index (HI) of heavy metals in *C. aurea* and *Z. scabra* leaves for an adult (65 kg, 64 years old) *.

Metal	*C. aurea* leaf samples			*Z. scabra* leaf samples			Hazard indices (HI)
	Concentration (mg/kg)	EDI (mg/kg/day)	THQ	Concentration (mg/kg)	EDI (mg/kg/day)	THQ	
Zn	0.5727	0.00088	0.0002	0.4547	0.00070	0.0002	0.0004
Pb	0.2394	0.00037	0.0062	0.2334	0.00036	0.0060	0.0122
Cd	0.0365	0.00006	0.0038	0.0468	0.00007	0.0048	0.0086
Cr	ND	-	-	ND	-	-	-

* ND = Not detected.

Table 13. Estimated daily intake (EDI, mg/kg/day), target hazard quotient (THQ), and hazard index (HI) of heavy metals in *C. aurea* and *Z. scabra* leaves for an adult (65 kg, 67 years old) *.

Metal	*C. aurea* leaf samples			*Z. scabra* leaf samples			Hazard indices (HI)
	Concentration (mg/kg)	EDI (mg/kg/day)	THQ	Concentration (mg/kg)	EDI (mg/kg/day)	THQ	
Zn	0.5727	0.00088	0.0002	0.4547	0.00070	0.0001	0.0003
Pb	0.2394	0.00037	0.0059	0.2334	0.00036	0.0057	0.0116
Cd	0.0365	0.00006	0.0036	0.0468	0.00007	0.0046	0.0082
Cr	ND	-	-	ND	-	-	-

* ND = Not detected.

The Cd levels in this study were comparable to those studied in Thailand [50], slightly higher than those in *Morus alba* leaves, but lower than those studied in *Marjorana hortensis* from Egypt [49].

3.9. Health risk assessment

Health risk assessment evaluates data related to human health, providing information on exposure to contaminants. For both children and adults, it uses metrics such as estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) to assess risks [52]. The EDI for metals in meals is calculated using average metal concentrations and consumption rates. The primary method for evaluating the health risks of metals in food involves estimating the levels of dietary intake and comparing them to established dietary standards and acceptable upper intake levels of the United States Food and Nutrition Board [53] for adults and the Joint Committee of Experts of the World Health Organization and the Food and Agriculture Organization on Food Additives [54-56] have established those values.

The estimated daily intake (EDI) of the individual metals resulting from the consumption of *C. aurea* and *Z. scabra* is ranked in the order of Cd < Pb < Zn. This indicates that Cd poses the least risk, followed by Pb, while Zn is the most prevalent. The EDI values obtained in this study were lower than the reference doses (RfD), suggesting that consuming these medicinal plants may not pose a health risk to consumers. Tables 12 and 13 summarize the target hazard quotients (THQ) and hazard indices (HIs) of metals in those plants. The THQ values for each metal were less than one in all samples, and the HI values ranged from 0.0003 to 0.0122. These results indicate that consumption of the medicinal plants studied likely does not pose a target risk quotient to the public, since the HI values are less than one. This suggests that the exposed population is unlikely to experience adverse health effects [57-60]. This highlights the importance of monitoring metal concentrations in these plants to protect public health while recognizing the potential nutritional benefits of Zn.

4. Conclusions

In this study, leaves of *C. aurea* and *Z. scabra* were collected from selected areas and Cr, Zn, Cd, and Pb were analyzed using FAAS. The recovery test, which uses an optimized sample processing procedure in the wet digestion method for both the medicinal plant samples *C. aurea* and *Z. scabra* investigation, gives a recovery percentage ranging from 88.2 to 95.8%, and 90.0 to 98.9%, the LOD of 0.0087-0.1476 and 0.0174-0.1176, respectively.. Metal concentrations in the *C. aurea* and *Z. scabra* leaves samples were Zn (0.5727 and 0.4547 mg/kg), followed by Pb (0.2394 and 0.2334 mg/kg), Cd (0.0365 and 0.0468 mg/kg in both samples, while Cr levels were below the detection limits. Health risk assessments were evaluated using estimated daily intake (EDI), target hazard quotients (THQ), and hazard indices (HI) related to the health risks caused by heavy metals in the two medicinal plants. The concentration values of all heavy metals in both samples were within the permissible limits established by the WHO, indicating that the concentration levels of these metals in medicinal plants are safe for human consumption and does not cause significant health risks. The health risk assessment values, including EDI, THQs and HIs, suggest that consuming these plants is unlikely to present a carcinogenic risk to local consumers, as the HI values for the analyzed samples were less than one. However, the continuous monitoring of the medicinal plants available on the market is essential to ensure that only safe and healthy products are offered for human consumption.

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Sample availability: Samples of the compounds are available from the authors.
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CRediT authorship contribution statement

Conceptualization: Negussie Megersa, Yihenew Keskise, Abi Legesse; Methodology: Yihnew Keskise, Negussie Megersa, Abi Legesse, Nibret Mekonen; Software: Yihenew Keskise, Nibret Mekonen, Abi Legesse, Tura Gemechu, Teshome Geahegne; Validation: Negussie Megersa, Abi Legesse, Nibret Mekonen, Teshome Gezahagne; Formal Analysis: Yihenew Keskise,, Abi Legesse, Nibret Mekonen, Teshome Gezahegne; Investigation: Negussie Megersa, Yihenew Keskise, Nibret Mekonen, Abi Legesse, Tura Gemechu; Resources: Negussie Megersa, Abi Legesse; Data Curation: Yihenew Keskise, Nibret Mekonen, Abi Legesse, Teshome Gezahagne; Writing - Original Draft: Negussie Megersa, Yihenew Keskise, Nibret Mekonen, Abi Legesse, Teshome Gezahagne; Writing - Review and Editing: Nibret Mekonen, Negusie Megersa, Abi Legesse, Teshome Gezahagne , Tura Gemechu; Visualization: Negussie Megersa, Yihenew Keskise, Nibret Mekonen, Tura Gemechu; Funding acquisition: Negussie Megersa; Supervision: Negussie Megersa; Project Administration: Negussie Megersa, Abi Legesse, Tura Gemechu.

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