

ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF CHEMISTRY



**DETERMINATION OF SOME HEAVY METALS IN TWO SELECTED MEDICINAL
PLANTS COLLECTED FROM SIDAMA REGION IN MASINCHO AREA**

BY

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September 12, 2024

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

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DECLARATION

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ACKNOWLEDGMENTS

Thanks and glory be to my creator, the almighty God without whom I couldn't move a jot of pace in this chaotic world which is full of terrible and horrible situations. It is his full protection and guidance which keep me safe and alive; and his direction amongst such terrifying and horrible situations which enabled me to arrive to this destination. Secondly I would like to thank my dear advisor Prof. Negussie Megersa for his earnest encouragements, skillful guidance and tirelessly revising the whole manuscript before being printed. In the same way, also I would like to thank two botanist doctors; Dr. Yadessa Gonfa and Dr. Firew Kebede from Hawassa University, without intimacy and acquaintance who kindly and earnestly replied my audacious questions to give me the taxonomy of the medicinal plants upon which I was conducting my research work.

Lastly I am very grateful to my colleagues and friends who kindly supported and encouraged me with their heartfelt advice and materials. Above all I am indebted to my staff members; Ato Amare Seid, Ato Michael Berhanu and Ato Ababayehu Haile who frequently asked and encouraged me with their collaborative ideas until I finish this work. Also my great thanks go to my friend Ato Mammo Cherinet, a senior chemistry teacher in Hawassa Tabor Senior Secondary School, for his encouraging and supportive ideas as well as material aids which helped me a lot in my work.

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Abbreviations and Acronyms

MTS	Traditional medicinal system.
CAM	Complementary alternative medicine.
TCM	Traditional chines medicines.
ITM	Indian traditional medicines.
AHM	Arabian herbal medicines.
AMM	Arabian materia medica.
ROS	Reactive oxygen species.
MFF	Metal fume fever.
HDL	High density lipoproteins.
LDL	Low density lipopprteins
HM	Heavy metal
WHO	World health organization

Abstract

In this study the collected samples of *P. vogelii* fruits and *R. myricoides* leaves were cleaned, dried in an oven and then ground to powder form. Then 0.5g of each powdered sample was digested with 4 mL of 69.72% of HNO₃ and 1 mL of 70% of HClO₄ at a temperature of 300°C for 4:00 hrs and 3mL of 69.72% HNO₃ and 1 mL of 70% HClO₄ at a temperature of 300°C for 3:00 hrs respectively. An efficient wet digestion procedure with the percentage recoveries which range from 99.33-99.97% for *P. vogelii* and 94.7-98.9% for *R. myricoides* were developed. The contents of heavy metals (Cu, Cr, Cd, Zn and Pb) in *P. vogelii* and *R. myricoides* samples were determined using FAAS and the result of mean concentrations of these metals were compared with the limit values set by WHO. The mean concentrations of the selected heavy metals were determined from triplicate samples of *P. vogelii* and *R. myricoides* herbal medicinal plants which were grown in Masincho area near Yirgalem town of Sidama region. In the present study, the mean concentrations of selected heavy metals analyzed in *P. vogelii* and *R. myricoides* samples in mg/kg were Zn (75.23 ± 0.038), Cu (17.68 ± 0.023), Pb (10.66 ± 0.0019), Cd (3.15 ± 0.0066) and Cr (BDL); and Zn (10.50 ± 0.0098), Cu (34.98 ± 0.006), Cd (8.70 ± 0.0063), Pb (18.60 ± 0.0072) and Cr (BDL) respectively. The contents of chromium were too low to be measured in both samples which were below detection limit and therefore, were not reported. The levels of these analyzed metals in this study are both higher and lower just like the concentrations of metals reported in different literatures in medical plant fruits and leaves.

Keywords: *P. vogelii*, *R. myricoides*, Heavy metals, world health organization and Flame atomic absorption spectroscopy.

1. INTRODUCTION

1.1 Background of the study

Traditional medicine is used throughout the world as it is dependent on locally available plants, which are easily accessible, and capitalizes on traditional wisdom repository of knowledge, simple to use and affordable. These medical systems are heavily dependent on various plant species and plant-based products [1]. Medicinal plants are plants, either growing wild or cultivated, used for their medicinal purposes [2]. They are consumed worldwide for the treatment of several diseases and such plants are also an important source of raw material for pharmaceutical industries [3]. In Ethiopia, up to 80% of the population uses traditional medicine. The major reasons why medicinal plants are demanded in Ethiopia are due to culturally linked traditions, the trust the communities have in medicinal values of traditional medicine, relatively low cost in using them and difficult access to modern health facilities [4, 5]. A common misconception is that medicinal plants are “pure and natural” and that this equates to “harmless”. Based on their long history of use, users of traditional medicines deem them safe for human consumption. However, the absence of their regulation provides no such guarantee [6]. Plants are contaminated with environmental pollutants especially heavy metals, which pose a great health risks to all living organisms upon long term exposures [7]. Cultivation in soils containing high concentrations of heavy metals is one mechanism of contamination of herbal products. Farmland, which may have been used for generations to produce medicinal herbs, may be directly encroached upon by factories, roads and other high pollutant areas or contaminated by aerosolized particles of waste material [8]. Emissions from heavy traffic on roads contain lead (Pb), cadmium (Cd), zinc (Zn) and nickel (Ni), which are present in fuel as anti-knock agents contaminate the nearby areas. Rivers and streams may also become contaminated and lead to downstream dispersion of heavy metals [6, 8]. Fertilizers, herbicides or insecticides containing heavy metals may also be applied in some settings. Many agro-chemicals contain As, Cu, Fe, Mn and Zn. Some contaminants such as Cd and Pb enter the soil as impurities in fertilizers. Especially Phosphate fertilizers represent a potentially significant source of Cd to soils. Therefore, the continued uses of contaminated fertilizers over an extended period of time cause accumulation of these contaminants to high levels in the soil [6]. Regardless of the source, plants are ultimately grown in soils with high concentrations of heavy metals. Plants absorb a number

of elements from this contaminated soil and accumulate them in their different parts. Heavy metals will get an access to human biological system as a result of using these contaminated medicinal plants to treat different ailments. Once being in human biological system, they will cause different health impacts [8, 9]. With the ever-increasing use of herbal medicines worldwide and the rapid expansion of the global market for these products, the safety and quality of medicinal plant materials and finished herbal medicinal products have become a major concern for health authorities, pharmaceutical industries and the public [2]. Different researches concentrating on the heavy metal content of medicinal plants have been conducted in various parts of the world and most of them showed that the levels of some heavy metals in these plants is higher than the maximum recommended limits set by different international organizations [10]. National regulation and registration of herbal medicines varies from country to country. Maximum allowable limit recommended by WHO are 1, 0.3 and 10 mg/kg for As, Cd and Pb in medicinal plants, respectively [2]. The safety and toxicity information of herbal medicine or products are required prior to expanded clinical studies and to support the registration of the herbal products with the Drug Control Agency. The presence of toxic heavy metals such as Cd, As, and Pb in herbal products at high levels pose serious risk to public health. These heavy metals when consumed in considerable amount can result in damaged or reduced mental and central nervous function, damage to blood composition, lungs, kidneys, liver, and other vital organs [11].

1.2 Statement of the Problem

Herbal medicines have been used for centuries in various cultures as a natural alternative to synthetic drugs, valued for their perceived safety and therapeutic benefits [12, 13]. In recent years, their use has surged globally, driven by the growing preference for natural health products. However, the increasing industrialization, environmental pollution, and lack of stringent quality control have led to concerns about the safety of these herbal medicines, particularly with respect to contamination by heavy metals [14, 15].

Heavy metals such as Pb, Cd, As, and Hg can contaminate herbal medicines through environmental exposure, including contaminated soil, water, and air, as well as during processing and storage [16]. Once accumulated in the human body, these metals can cause serious health

risks, including neurotoxicity, carcinogenicity, renal damage, and cardiovascular issues, particularly through prolonged use or consumption above safe limits [12, 13]. This problem is exacerbated by the fact that many consumers and even practitioners of traditional medicine are unaware of these potential contaminants.

Despite the widespread use of herbal medicines, there is a significant gap in regulatory frameworks for monitoring and controlling heavy metal contamination in many countries, especially in developing regions. The World Health Organization (WHO) has issued guidelines urging the testing of herbal products for contaminants, including heavy metals, to ensure consumer safety [16]. However, implementation of these guidelines remains inconsistent. A study reported in scientific literature [17] revealed that many herbal medicines, particularly those marketed in developing regions, are not routinely tested for heavy metal contamination.

The absence of regular testing and enforcement of safety standards contributes to the risk of toxic exposure, particularly for vulnerable populations such as pregnant women, children, and those with compromised immune systems. Given that herbal products are often perceived as “natural” and “safe,” consumers might inadvertently expose themselves to harmful levels of heavy metals over time, leading to adverse health outcomes [14, 15]. Therefore, it is mandatory to assess toxic metal concentration in commonly used medicinal plants to take a corrective action. Though similar studies have been conducted in different parts of the world, there are very limited reports on heavy metal content of medicinal plants originated from Ethiopia. To the best of the investigator’s knowledge, no report on the heavy metal content of medicinal plants used in Sidama area district. This work was done to measure the level of five metals (Cd, Cr, Pb, Cu, and Zn) in two selected medicinal plants.

The purpose of this study is, therefore, to determine the concentration of heavy metals in selected herbal medicines and evaluate their safety for human consumption. This research work is aimed to fill the gap by providing quantitative data on the levels of heavy metals in herbal traditional medicine, contributing to the development of more robust quality control measures and regulatory standards.

1.3 Objectives of the study

1.3.1 General objective

The main purpose of this study is to determine the level of selected heavy metals in two medicinal plants collected from Masincho area located around south of Yirgalem town in Sidama region.

1.3.2 Specific objectives

- To investigate the concentration of heavy metals in the two selected medicinal plants of Sidama region.
- To clarify the side effects of using these medicinal plants.
- To compare the variations of the heavy metal concentrations between the selected herbal medicines Surpa (*Peponium vogelii*) and Butamo (*Rutheca myricoides*).
- To compare the levels of mentioned heavy metals in herbal plants with other herbal plants from literatures.
- To provide baseline information and make recommendation for the future on these herbal plants.

1.4 Significance of the Study

The main purpose of this study is to determine the concentration of selected heavy metals in the medicinal plants upon which the society living in the area, at large are highly dependent. It is obvious that medicinal plants have not only advantages but also disadvantages, which are not clearly known by the African people in general. Therefore, in this study efforts have been made to point out some side effects of medicinal plants that the people living in the vicinity have to know. One of the principal roots of these side effects is mentioned as the concentration of toxic heavy metals; although there are several other toxic chemicals like cyanogenic glycosides and many others which are found in some medicinal plants and cause adverse effects. Therefore, from the results of this work, it is hoped that the concerned bodies will take firm actions which warn and prevent people from excessive use of these medicinal plants.

Furthermore, the study will raise awareness among consumers, practitioners, and regulatory authorities about the potential risks of heavy metal contamination in herbal medicines and the need for regular testing to safeguard public health.

2. LITERATURE REVIEW

2.1 Traditional Herbal plants

The use of traditional herbal medicines is a widespread practice in Ethiopia, with over 80% of the population relying on these natural remedies for their primary healthcare needs [18]. Ethiopia's rich biodiversity and the cultural significance of medicinal plants have made herbal medicine a cornerstone of healthcare, particularly in rural areas. However, the increasing concerns over heavy metal contamination in these plants raise critical questions about their safety. Heavy metals, including Pb, Cd, As, and Hg, are known to accumulate in medicinal plants, often due to environmental pollution, agricultural practices, and poor-quality control in harvesting and preparation [19]. This review examines the studies conducted on the presence of heavy metals in traditional herbal plants in Ethiopia, focusing on the sources, health risks, and mitigation measures.

Sidama region is one of the naturally endowed Ethiopian regions with a multitude of biodiversity and different topographic landforms ranging from lowlands to highlands and different climatic conditions. Due to this gift of nature the region's flora is estimated to 85% plants from which about 40% are assumed to be medicinal herbal plants. In sidama region every type of disease whether it is that of humans or domestic animals has its own remedy by sidama herbalists. The ailments which do not have remedies in conventional medicines such as hepatitis, HIV/AIDS and even corona are reported to be cured by these traditional medicine practitioners [20, 21,22].

The preparation as well as the administration of these traditional medicines is similar with that of other Ethiopian traditional medicine practitioners in particular and that of Africans in general [23, 24, 25].

Because of the differences in altitude and climatic conditions as well as the differences in the type of soil, different types of herbal plants with different properties are grown in this region. As it was described earlier, there might be a large number of other herbal plants which are considered as ordinary simple plants, but which could be used as medicines somewhere in other communities. Including these plants we could dare to say that almost about half percent of Sidama's flora comprise medicinal plants. As it is common in other Ethiopian communities, most of Sidama

population live in rural areas and rely on their reputable traditional herbal medicines for their wellbeing and the health of their domestic animals. In all over the world, in communities who overwhelmingly rely on traditional medicines for their health care, it is customary that spiritual practices like shamanism play a large part. In the same way the vast majority of Ethiopian traditional healers including those of Sidama region, integrated their knowledge of traditional medicines with spiritual practices [22, 25].

Many researches so far conducted in Sidama region on medicinal plants have shown that most medicinal plants are used to treat only single illness and some were reported to have medicinal roles for more than two or more illnesses [20, 26]. For example, *Peponium vogelii*, one of the key points of this study is a broad spectrum medicinal plant used to treat various human ailments. In one research conducted in Boricha district of Sidama region, from the collected 57 medicinal plants, 36 or 63.2% were found to be used to treat only human ailments; 6 or 10.5% were found to be used to treat only livestock ailments and 15 medicinal plants i.e. 26.3% were found to be used to treat both human and livestock ailments [26]. Among the most common medicinal plants used by the Sidama community, are *peponium vogelii* whose name in vernacular language is Surpa and *Rotheca myricoides* whose name in vernacular language is Butamo; are notable and famous medicinal plants.

Peponium vogelii is a herbaceous perennial climbing plant which produces steps up to 10 meters long that scramble over the ground or climb into nearby vegetation, supporting themselves by means of tendrils. *Peponium* is a genus of flowering plants which belongs to cucurbitaceae family which has 130 genera and has about 800 species. *Peponium vogelii* is the species distributed mainly in tropical and subtropical regions of the world. The three most common genera of this species are:

1. Cucmis (cucumbers, melons)
2. Cucurbita (pumpkins, squash)
3. Citrulhus (water melons)

These genera are among the top ten economically important vegetable crops of the world while several other members have regional importance. The fruits, seeds and leaves of some cucurbitaceae species are traditionally consumed in various ayurvedic preparations and confectionaries [27, 28].



Figure 1. A Trailing, a Climbing and a fruit of *Peponium vogelli* plant.

Rothea is a genus of flowering plants in the lamiaceae family. Lamiaceae is the mint family of flowering plants which has 236 genera and more than 700 species. The number of species belonging to *Rothea* genus is estimated to about 35 to as many as 60; one of which is *Rothea myricoides* ugandense which is commonly known as butterfly bush due to the resemblance of its flowers with flying butterfly. This lanky shrub grows to 6-10 feet and has light blue to violet blue pea like flowers and bears four lobed (6-10mm) fruits. *Rothea myricoides* ugandense is a cultivar originally called clerodendrum ugandense which was later changed into clerodendrum myricoides ugandense. The current name *Rothea myricoides* ugandense was applied based on the result of DNA studies [29, 30].

Rothea myricoides is the species which is native to sub-saharan Africa, i.e in tropical east Africa starting from Eritrea and extends up to South Africa, [31, 32] while *Rothea serrate* is the species which is native to Asia, particularly to India, Srilanka and Malayasia.



Figure 2. *Rothea myricoides* plant without and with its flowers.

2.2 Heavy Metals

The term Heavy Metals refer to any metallic element that has a relatively high density and is toxic or poisonous at low concentration [33]. Heavy Metals are a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3 , or 5 times or more, greater than water [34, 35 ,36] and also Heavy Metals are defined as those elements with a specific density at least five times the specific gravity of water, Heavy Metals include Cd, Cu, Pb, Zn, Hg, As, Ag, Cr, Fe and Platinum group elements, Cu and Zn are essential trace elements for living organisms at low concentration (10 mg/L). Most of these metal ion (Cd, Cu, Zn, Hg, As, Ag, Cr and Fe) can be released from the industries are in simple cationic forms [37]. The characteristics of heavy metals are described by Wang [38]. Toxicity can last for a long time in nature. Heavy Metals cannot be degraded including bio treatment and are very toxic even at low concentration (1.0-10.0 mg/L).

Heavy Metals are 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 chemicals concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (Metabolized) excreted [39]. World Health Organization (WHO) has established levels of metals in foods above which, they should not be consumed. For this reason, the levels of trace metals in our food should be of much importance and concern to us.

2.2.1 The Selected heavy metals for this study

Heavy metals like Cu, Zn, Cr, Cd, and Pb are commonly found in the environment and can accumulate in medicinal plants used as herbal medicines. While Cu and Zn are essential trace elements, Cd, Pb, and Cr (in its hexavalent form) pose significant health risks when present in excess. The World Health Organization (WHO) has set guidelines to limit human exposure to these metals in herbal medicines to ensure safety.

Copper (Cu): Copper is an essential nutrient involved in various biological functions such as enzyme activity, iron metabolism, and antioxidant defense. However, high concentrations of copper in herbal plants can lead to toxicity.

Health Risks: Excessive copper intake can cause gastrointestinal symptoms such as nausea, vomiting, and diarrhea. Long-term exposure can lead to liver and kidney damage, anemia, and neurotoxicity [39].

WHO Recommendations: WHO has recommended a permissible limit of 20 mg/kg in medicinal plants. The tolerable upper intake level (UL) for copper in adults is 10 mg/day [41].

Zinc (Zn): Zinc is essential for immune function, wound healing, and protein synthesis. However, excessive amounts in herbal products can cause adverse effects.

Health Risks: Symptoms include nausea, vomiting, cramps, and headaches. Over time, high zinc levels can interfere with copper absorption, leading to a copper deficiency. It can also suppress the immune system and cause neurological problems [42].

WHO Recommendations: The permissible limit for zinc in medicinal plants is 50 mg/kg . The tolerable upper intake level for zinc in adults is 40 mg/day [42].

Chromium (Cr): Chromium is found in two forms: trivalent chromium (Cr (III)), an essential nutrient, and hexavalent chromium (Cr (VI)), a toxic form. Trivalent chromium in small amounts is needed for glucose metabolism, but Cr (VI) is highly toxic and carcinogenic.

Health Risks: High levels of Cr (VI) cause gastrointestinal distress, skin irritation, and respiratory problems. Prolonged exposure to Cr (VI) can cause lung cancer, liver and kidney damage, and dermatological issues [43].

WHO Recommendations: The WHO recommends a permissible limit of 1.30 mg/kg for chromium in medicinal plants [41]. No tolerable upper intake level for Cr (III) has been set, but Cr (VI) should be avoided in herbal plants.

4. Cadmium (Cd): Cadmium is a non-essential metal that is toxic even at low concentrations. It can accumulate in the liver and kidneys, posing severe health risks. Gastrointestinal issues such as nausea, vomiting, and abdominal pain. Long-term exposure to cadmium causes kidney damage, bone fragility (osteomalacia and osteoporosis), and an increased risk of cancer [44].

WHO Recommendations: The permissible limit for cadmium in medicinal plants is 0.2 mg/kg [45]. The tolerable monthly intake (TMI) for cadmium is 25 µg/kg body weight [40].

Lead (Pb): Lead is highly toxic and poses significant health risks even at low exposure levels. It has no known beneficial role in the human body and can accumulate in bones and tissues.

Health Risks High lead exposure can cause abdominal pain, vomiting, and seizures. Long-term lead exposure can result in neurological damage, particularly in children, causing cognitive impairment, learning disabilities, and developmental delays. In adults, it can lead to kidney damage, hypertension, and reproductive issues [46].

WHO Recommendations: The permissible limit for lead in medicinal plants is 2 mg/kg (WHO, 2007). There is no safe level of lead exposure, and efforts should be made to minimize lead in herbal medicines [45].

2.2.2 Heavy Metal Contamination in Herbal Plants

Heavy metals can accumulate in medicinal plants due to various environmental and anthropogenic factors. Soil contamination from industrial waste, mining activities, fertilizers, and pesticides are the primary sources of heavy metals in Ethiopia [47]. For example, the Addis Ababa area and other urban centers have seen increased soil and water pollution from industrial activities, affecting nearby agricultural and medicinal plant fields. In particular, plants such as *Hagenia abyssinica* and *Thymus schimperi*, which are widely used in Ethiopian traditional medicine, have shown heavy metal accumulation when grown near contaminated sites [48].

Research by [49] investigated heavy metal concentrations in commonly used medicinal plants collected from various regions of Ethiopia. The study found that the levels of lead and cadmium in some plants exceeded the permissible limits set by the World Health Organization (WHO). In

some cases, these plants were found to have absorbed heavy metals from contaminated soil or irrigation water, raising concerns about their long-term safety for human consumption.

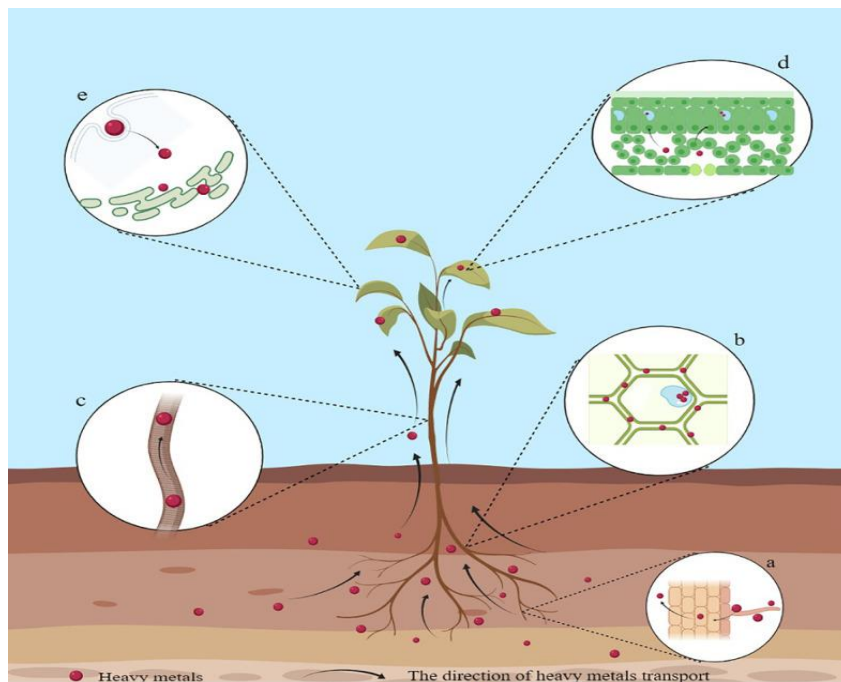


Figure 3. Schematic diagram of the mechanism of HM accumulation in plants. (A) Accumulation of HMs on the surface of the root system; (B) Accumulation of HMs in cell walls; (C) Root-to-shoot translocation; (D) Accumulation of HMs in leaves; (E) Endocytosis [50].

2.2.3 Sources of Heavy Metals contamination in Ethiopia

In Ethiopia, heavy metal contamination in herbal plants often results from environmental pollution linked to mining, industrial activities, and the use of agrochemicals. For instance, a study by [51] highlighted the problem of heavy metal contamination in areas around the Akaki river, which runs through Addis Ababa. The river is a major source of irrigation for local agriculture, including medicinal plant farms, and has been heavily polluted by industrial waste, leading to increased levels of lead, mercury, and arsenic in the soil and plants.

The use of traditional methods in the collection and preparation of medicinal plants also contributes to contamination. Traditional healers often gather plants from areas near roadsides or

industrial sites, where the risk of exposure to pollutants is higher [52]. In addition, improper drying and storage methods may further contribute to contamination, especially when herbs are exposed to dust and heavy metals during handling and transportation [53].

2.2.4 Health Risks Associated with Heavy Metals

The presence of heavy metals in medicinal plants poses significant health risks to consumers, particularly with long-term use. Lead and cadmium are particularly harmful, as they can accumulate in the body over time and cause various chronic health problems, including kidney damage, neurological disorders, and cancer [54]. For example, lead exposure can impair cognitive development in children and cause cardiovascular issues in adults [44].

Studies on Ethiopian herbal medicine users have highlighted concerns about these risks. A survey by [53] found that many consumers of traditional medicine were unaware of the potential dangers posed by heavy metal contamination. Given that herbal medicines are often taken over long periods, the risk of chronic exposure to toxic metals is significant. These health risks underscore the need for stricter quality control and regulation of medicinal plants in Ethiopia to protect public health.

2.2.5 Mitigation measures and regulatory framework

Addressing the issue of heavy metal contamination in herbal medicines in Ethiopia requires a multifaceted approach. The World Health Organization (WHO) has established guidelines for assessing the quality of herbal medicines, particularly in relation to contaminants such as heavy metals [45]. However, Ethiopia lacks a comprehensive regulatory framework to enforce these guidelines and ensure that traditional herbal products are safe for consumption.

Some efforts have been made to improve the quality control of medicinal plants in Ethiopia. For instance, [48] recommended routine monitoring of soil and water quality in areas where medicinal plants are cultivated, along with better training for traditional healers on safe harvesting and preparation practices [51] also called for increased public awareness of the risks associated with contaminated herbal products and the importance of sourcing plants from uncontaminated areas.

2.3 Analytical methods for determining heavy metals in herbal plants

The determination of heavy metals in herbal plants requires accurate and sensitive analytical techniques due to the potential health risks posed by these contaminants. Several methods are used to detect and quantify heavy metals such as Cu, Zn, Cr, Cd, and Pb in herbal plants. Below is a detailed overview of the most commonly employed analytical methods, along with references.

2.3.1 Microwave Digestion

This digestion method is faster, cleaner, more reproducible and more accurate than acid digestion method. It uses microwave radiations to speed up the decomposition and extraction of the required mineral elements. Microwave energy, as a heat source for sample digestion was first described at the beginning of 19th century. In principle, samples or solvents containing dipolar materials or microwave absorbents can be affected by microwaves which heat the substance being digested from inside to outside in a very short period of time in a much different way from the common heating system. Microwave digestion also involves either open or closed system. From these, the closed system offers a special way of regulating the extraction temperature [55, 56].

2.3.2 Dry Decomposition or Dry Ashing

This method is the simplest and the easiest way of digesting; which is performed by placing the sample matrix in an open inert vessel to eliminate organic compounds by ignition in air or in a stream of oxygen. Usually additives like $\text{Mg}(\text{NO}_3)_2$ and H_2SO_4 are used to avoid losses of volatile elements such as As, Cd, Hg and Pb. Dry ashing is performed in a muffle furnace capable of maintaining the ashing temperature range of 450-550⁰C in the presence of ashing acid. H_2SO_4 , water and volatiles are vaporized and the organic substances are burned to form CO_2 and oxides of nitrogen. Most mineral elements are converted into their oxides, sulfates, phosphates, chlorides and silicates which are not volatilized. The ash is then dissolved in HCl for further analysis by

spectroscopic methods. The elements Ca, Mg, Fe, Zn, Cu and Mn are easily determined by this method [55, 57].

2.3.3 Acid Digestion or Wet Decomposition

This method involves the utilization of mineral or oxidizing acids and heat from an external source to decompose the sample matrix. The choice of acid or combination of acids depends on the nature of the matrix to be decomposed. Wet decomposition has been practiced in open systems for many years, because they are useful for relatively simple samples such as food, but they are not generally suitable for samples that require a long period of time, 1-24 hours. This means they are time consuming and tedious. They also cause evaporative loss of volatile metals and risk of contamination.

In general HNO_3 is used as oxidant alone or in combination with other acids such as HCl , HClO_4 or sometimes with H_2O_2 , to increase its oxidizing power. H_2SO_4 alone or with HNO_3 is not usually recommended as a digestion reagent due to its ability to form insoluble salts with some metals such as Ba, Sr, Hg, and Pb. In samples containing Ag or Pb, HCl also forms insoluble salts; therefore, in its stead, HClO_4 or H_2O_2 may be used as a digesting reagent. HClO_4 is extremely strong oxidizer, that it reacts violently or explosively to oxidize organic compounds and attacks nearly all metals.

Because of its some limitations, instead of open system digestion; closed system digestion is used to improve the oxidation efficiency and to reduce the time of digestion. Closed systems allow high pressures above atmospheric pressure which causes boiling at elevated temperatures and often tends to complete the dissociation of most samples. It also reduces contamination from external sources and prevents the volatilization of volatile elements. It was this method that has been used in this study for the digestion of sample matrices [55, 58].

2.3 Instrumentation for Heavy Metal Analysis

I. Atomic Absorption Spectroscopy (AAS)

AAS is one of the earliest commercially developed methods for the elemental analysis of HMs [59]. AAS is an approach for quantitative analysis based on the absorption of characteristic spectral lines by atomic vapor produced from a substance. It comprises five main components: a light source, an atomization system, a spectroscopy system, a detection system, and a display unit. The operating principle is as follows: First, an atomizer turns the sample to be measured into an atomic vapor under high temperature. Next, when the atomic vapor is irradiated with a light source, it can absorb light radiation of a certain wavelength. After this, the spectroscopic system distinguishes between different spectral lines. Finally, the content of the element to be measured in the sample is determined according to the degree of attenuation of the light when it is absorbed. AAS possesses the advantages of high selectivity, accuracy, sensitivity, and low interference's ability. The equipment is easy to operate, enables fast analysis, and provides an extensive range of analyses [60]. Depending on the atomization device used, AAS can be categorized into cold vapor generation atomic absorption spectrometry (CVAAS), hydride generation atomic absorption spectrometry (HGAAS), graphite furnace atomic absorption spectrometry (GFAAS), and flame atomic absorption spectrometry (FAAS) [61, 62, 63].

Principle: AAS measures the absorption of light by free atoms in the gaseous state. The plant sample, after digestion, is atomized, and a specific wavelength of light corresponding to the metal of interest is passed through the sample. The amount of light absorbed is proportional to the concentration of the metal in the sample [64].

A. Flame Atomic Absorption Spectroscopy (FAAS)

For the determination of essential and toxic trace metals in traditional herbal plants different spectrochemical methods are used. However, FAAS is one of the most extensively used techniques for discriminating various elements with significant precision and accuracy. The main advantages of FAAS with atomization in a widespread air acetylene flame are low operational costs and reasonably good analytical performance [65, 66]. The possibility of only sequential analysis and the narrow ranges of linear response can be regarded as disadvantages of this method. Using FAAS for the determination of metals in traditional herbal plants the samples are usually mineralized in order to avoid ion interference matrices. This method is suitable for

detecting metals like Cu and Zn. It involves spraying the digested sample solution into a flame, where it is atomized. FAAS is sensitive but less effective for trace metals like Cd and Pb (67).

B. Graphite Furnace AAS (GFAAS)

GFAAS is a more sensitive version of AAS, suitable for trace metals like Cd and Pb. It atomizes the sample in a heated graphite tube instead of a flame, allowing for better detection limits [68].

Advantages: AAS offers high accuracy, precision and relatively simple sample preparation.

Disadvantages: It can only measure one element at a time and has limited detection for very low concentrations of certain metals.

2.5 Quality Control and Validation

Calibration: Calibration curves using standard solutions of known metal concentrations are used to ensure accuracy.

Blank Samples: Blank samples without metals are analyzed to check for contamination.

Reference Materials: Certified reference materials (CRM) are analyzed alongside plant samples to validate the accuracy of the results [69].

Recovery Tests: Spiked samples (with known concentrations of metals) are analyzed to verify the accuracy of the sample preparation and analytical procedure.

3. EXPERIMENTAL

3.1. Apparatus and equipment

When conducting this study clean ethylene bags bought from a shop were used to collect the medicinal plant leaves and fruits; a drying oven was used to dry the collected leaves and fruits and ceramic mortar and pestle were used to grind the dried samples. Then plastic sieves were used for sieving the ground sample powder which was kept in a clean container until digestion process. Digital analytical balance was used to weigh the samples. Measuring cylinders to measure different volumes of acid reagents, sample solutions and standard solutions were used.

Gallephamp Kjeldahl apparatus was used to digest the sample solutions and the digestion process was performed in the lab fume hood. During the operation, 100 mL round bottomed flasks fitted with reflux condensers were used in the Kjeldahl apparatus for the digestion process. 50 mL volumetric beakers, pipettes, funnels, wash bottles safety gloves, spatulas and detergents were used during digestion process.

ZEE nit 700, a compact tandem flame atomic absorption spectrometer with automatic flame mode was used to determine the heavy metals in the sample solution

3.1.1 Cleaning apparatus and chemicals

Before the experimental procedure starts, all the experimental apparatus and equipments are thoroughly washed and cleaned with tap water and then with deionized water in order to avoid some foreign particles and other possible contaminations.

3.2. Reagents and chemicals

The reagents used in the analysis of this study were all analytical grade. HNO_3 (69.72%) and HClO_4 (70%) for digestion of the samples. Apart from the reagents used, distilled water was used both for diluting the concentrated acids and for rinsing the beakers, flasks and test tubes.

Standard solutions containing 1000 g/L; in 2% HNO_3 of metals Cd, Pb, Cr, Zn and Cu were used for the preparation of calibration of standards and spiking experiments.

3.3. Sample area description

This study was conducted in one of the regions found in the southern part of Ethiopia called Sidama region. In this region, randomly Masincho area near Yirgalem town was selected as the site of the study; because all Sidama people culturally use all types of herbal medicinal plants for their health care as well as for the wellbeing of their livestock.

The types of medicinal plants were also randomly chosen, because all types of medicinal plants are available everywhere, although some are known by traditional medicine practitioners and are concealed from the society for the sake of business.

The samples were collected from the banks of flowing woyima brook located south of Yirgalem town, and from Berra and Masincho kebeles, which are south of the flowing woyima brook.

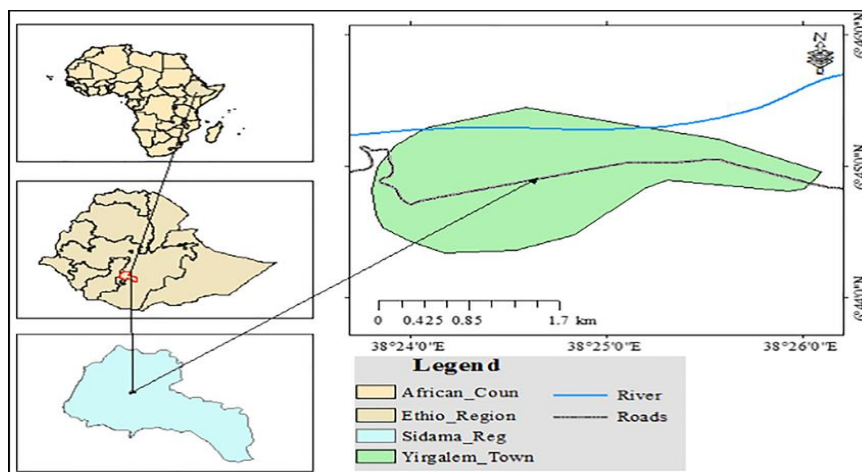


Figure 4. Location of sampling area

3.4. Sample preparation

Prior to heavy metals analysis in samples by different spectroscopic methods, the organic sample matrices require the total dissolution and digestion, because all analytical spectroscopic methods always need a simple or complex sample preparation. This preparation allows the separation and pre concentration of analytes before the analysis of the heavy metals, because 30% of the total analysis error lies in these steps. Nowadays, the goals to be achieved during analysis are the best results within the shortest time, with a limited amount of reagent and energy consumption followed by the least mineral contamination and production of little waste or residue.

In this study before digestion process, the completely washed and cleaned leaves and fruits of the medicinal plants were spread on a polyethylene sheet to be dried in an open air. Then for complete drying, again they are placed in an oven on a sheet of aluminum foil at 70°C for 24 hours. After they had been completely dried, they were taken out of the oven to be crushed and ground into powder form. Then to obtain a more fine powder, the ground powder was sieved with plastic sieves having holes of about 0.25 mm diameter. Then the powdered sample was kept in a thoroughly cleaned vial until the digestion was started.

3.5. Optimization procedure of digestion of samples

Prior to digestion of sample matrices, digestion parameters such as temperature, volume and time have to be optimized. This procedure was performed by trial and error method by successively increasing and decreasing the temperature, volume and the time; until the minimum temperature, volume and time at which clear solution is formed is obtained. The optimization procedure saves the wastage of the amount of the volume of reagents and the energy used as well as the time required for digesting the sample matrices. Once the optimum temperature, volume and time at which clear solution is obtained were known, the whole digestion process was carried out at these conditions.

During digestion process all volatile organic substances are volatilized and a clear solution containing only the required metals will be left over. It is this clear solution that is introduced into FAA spectrometry machine to determine the sought metals.

Table 1. Different conditions tested for optimization of digestion procedure for 0.5 g samples of *Peponium vogelii* fruit medicinal plant.

I. Volume optimization

Serial No	Reagents	Volume in mL	Temperature in °C	Time in hour	Observation
1	HNO ₃ :HClO ₄	3:1	300	3:00	Greenish yellow
2	HNO ₃ :HClO ₄	3:2	300	3:00	Pale yellow
3	HNO ₃ :HClO ₄	4:1	300	3:00	Clear solution
4	HNO ₃ :HClO ₄	4:2	300	3:00	Whitish clear
5	HNO ₃ :HClO ₄	4:3	300	3:00	Pale yellow
6	HNO ₃ :HClO ₄	5:1	300	3:00	Greenish yellow

II. Temperature optimization

Serial No	Reagents	Volume in mL	Temperature in °C	Time in hour	Observation
1	HNO ₃ :HClO ₄	4:1	150	4:00	Greenish yellow
2	HNO ₃ :ClO ₄	4:1	180	4:00	Greenish yellow
3	HNO ₃ :HClO ₄	4:1	210	4:00	Pale yellow
4	HNO ₃ :HClO ₄	4:1	240	4:00	Pale yellow
5	HNO ₃ :HClO ₄	4:1	270	4:00	Whitish clear
6	HNO ₃ :HClO ₄	4:1	300	4:00	Clear solution

III. Time optimization

Serial No	Reagents	Volume in mL	Temperature in	Time in hour	Observation
1	HNO ₃ :HClO ₄	4:1	300	1:30	Greenish yellow
2	HNO ₃ :HClO ₄	4:1	300	2:00	Greenish yellow
3	HNO ₃ :HClO ₄	4:1	300	2:30	Pale yellow
4	HNO ₃ :HClO ₄	4:1	300	3:00	Pale yellow
5	HNO ₃ :HClO ₄	4:1	300	3:30	Whitish clear
6	HNO ₃ :HClO ₄	4:1	300	4:00	Clear solution

Table 2. Different conditions tested for optimization of digestion procedure for 0.5 g samples of *Rotheca myricoides* leaf medicinal plant.

I. Volume optimization

Serial N o.	Reagents	Volume in mL	Temperature in	Time in hrs	Observation
1	HNO ₃ :HClO ₄	2:1	270	3:00	Pale yellow
2	HNO ₃ :ClO ₄	2:2	270	2:00	Pale yellow
3	HNO ₃ :ClO ₄	3:1	270	2:00	Clear solution
4	HNO ₃ :ClO ₄	4:1	270	2:00	Pale yellow
5	HNO ₃ :ClO ₄	4:2	270	2:00	Whitish clear
6	HNO ₃ :HClO ₄	5:1	270	2:00	Greenish yellow

II. Temperature optimization

Serial No	Reagents	Volume in mL	Temperature in °C	Time in hour	Observation
1	HNO ₃ :HClO ₄	3:1	150	2:00	Pale yellow
2	HNO ₃ :HClO ₄	3:1	180	2:00	Pale yellow
3	HNO ₃ :HClO ₄	3:1	210	2:00	Pale yellow
4	HNO ₃ :HClO ₄	3:1	300	2:00	Clear solution
5	HNO ₃ :HClO ₄	3:1	270	2:00	Whitish clear
6	HNO ₃ :HClO ₄	3;1	240	2;00	Coudy

III. Time optimization

Serial No	Reagents	Volume in mL	Temperature in °C	Time in hour	Observation
1	HNO ₃ :HClO ₄	3:1	300	1:00	Pale yellow
2	HNO ₃ :HClO ₄	3:1	300	1:30	Pale yellow
3	HNO ₃ :HClO ₄	3:1	300	2:00	Coudy
4	HNO ₃ :HClO ₄	3:1	300	2:30	Whitish clear
5	HNO ₃ :HClO ₄	3:1	300	3:00	Clear solution
6	HNO ₃ :HClO ₄	3:1	300	330	Whitish clear

3.6. Digestion of samples

After the optimization parameters were optimized, 0.5 g sample of each medicinal plant was weighed with analytical balance and under similar conditions of optimization, each sample was digested in triplicate. During the digestion process, as it was done for optimization, 0.5 g of each sample was added into each three 250 volumetric round bottomed flasks for each medicinal plant, and HNO_3 and HClO_4 in the volume ratio of 4:1 (for peponium vogelii triplicate samples) and 3:1 (for rotheca myricoides triplicate samples) were added. Then the samples were digested with Kjeldahl heating apparatus for 300°C for 4 hours (for peponium) and for 3 hours (for rotheca). After clear solutions were formed the temperature was switched off and after 20 minutes, the clear solutions were filtered through Whatmann filter paper into 50 mL volumetric flask which was already cleaned with deionized water. Then into each clear solution in the 50 mL flask, deionized water was added until they are filled up to the marked level. Then each sample solution was labeled and kept in refrigerator. The blank solutions were also prepared in the same way in triplicate for each sample and were kept with sample solutions in the refrigerator until they were introduced into FAAS machine for the determination of heavy metals.

3.7. Determination of heavy metals

From standard stock solution, secondary standard solutions were prepared by diluting it with deionized water and from these secondary solutions four standard working solutions for each metal of interest were prepared to calibrate the instrument. After calibrating the instrument, the already prepared clear sample solutions were introduced into the FAAS machine and the concentration of each metal in sample solutions was determined. Triplicate analysis was carried out for each metal in each sample solution to determine the average concentration of each metal in sample solutions. From the results obtained from FAAS; MDL and MQL were determined.

3.7.1 Method detection limit (MDL)

MDL is the lowest amount of analyte in a sample solution which can be detected but not necessarily quantitated as an exact value. It is obtained by multiplying the standard deviation of the reagent blank by 3 [70].

$$MDL=3 \times SD_{\text{blank}} \dots\dots\dots (1)$$

In this study six blank samples were digested following the same steps and procedures as that of sample solutions. The standard deviation was calculated for each metal from the six blank measurements to determine the MDL for each metal of interest. As the analyte level approaches the MDL, the uncertainty of the measurement increases dramatically and precision starts to decrease.

3.7.2 Method quantitation limit (MQL)

MQL is the lowest concentration of the analyte in a sample solution that can be quantitated with a suitable accuracy and precision as an exact value [71]. It has high significance when the intended analytical method is determining some particles or impurities in the given sample solutions. It is obtained by multiplying the standard deviation of the reagent blank by 10.

$$MQL = 10 \times SD_{\text{blank}} \dots\dots\dots (2)$$

Table 3. Instrument operating conditions for determining heavy metals in *P. vogelii* and *R. myricoides* using FAAS.

Metals	$\lambda(\text{nm})$	SW(nm)	LC(mA)
Zn	213	0.7	2.0
Cu	324	0.7	1.5
Cr	357	0.7	2.0
Cd	228	0.7	2.0
Pd	283	0.7	2.0

3.7.3. Method validation

Method validation is a process used to confirm that the steps and procedures employed for a specific test are suitable for their intended purpose. In this study the analytical parameters for the determination of heavy metals in the sample solutions were evaluated by the linearity of correlation coefficients, MDL and MQL results.

3.7.4 Recovery test for the optimization procedure

Recovery tests are techniques used for validating the performance of analytical methods. Recovery studies involve the addition of a known amount of analyte to a sample and then determining what percent of the amount added is detected. These experiments are conducted to estimate proportional systematic errors whose magnitude increases with the concentration of analytes [72]. The error is often caused by a substance in the sample matrix that reacts with the sought analyte; and therefore competes with the analytical reagent. Percent recovery is calculated by the following formula.

$$\% \text{Recovery} = \frac{\text{concentration of spiked sample} - \text{concentration of unspiked sample}}{\text{concentration of standard solution added for spiking}} \times 100$$

The efficiency of the methods used in this work was assessed by spiking a known concentration of each metal in to 0.5g of each sample and analyzed in triplicate.

4. RESULTS AND DISCUSSION

4.1 Instrument calibration

The reliability of results obtained from the analysis of FAAS depends on the calibration and standard solution preparation. For example, calibration curves show good linearity with low concentration standard solutions, but curves with highly concentrated solutions deviate from linearity. Therefore, it is essential to use low concentration standard solutions. Thus, a proper instrument calibration and an authentic standard solution preparation result in reliable output of the instrument from which the desired amounts of the unknown metals are determined.

The concentration of stock solution, intermediate standard solution and working standard solution, calibration curves obtained from the results of FAAS and their corresponding correlation coefficients are shown as follows in Table 4.

Table 4. The concentration of stock solution, intermediate standard solution, working standard solution and the corresponding correlation coefficient and equation of calibration curve.

Heavy metals	Conc. of stock Solution(mg/L)	Concentration of intermediate standard solution (mg/L)	Concentration of working standard solution (mg/L)	Correlation coefficient (R)	Equation for calibration curves
Zn	1000	10	0,0.25, 0.5, 0.75, 1.0	0.9985	$a=0.5709c + 7.9 \times 10^{-3}$
Cu	1000	10	0,0.25, 0.5, 0.75, 1.0	0.9978	$a=0.0716c - 1 \times 10^{-3}$
Cr	1000	10	0,0.25, 0.5, 0.75, 1.0	0.9981	$a=0.0716c - 1 \times 10^{-3}$
Cd	1000	10	0,0.25, 0.5, 0.75, 1.0	0.9979	$a=0.0864c + 6 \times 10^{-4}$
Pb	1000	10	0,0.25, 0.5, 0.75, 1.0	0.9996	$a=0.0327c + 6.9 \times 10^{-3}$

(a) Zn

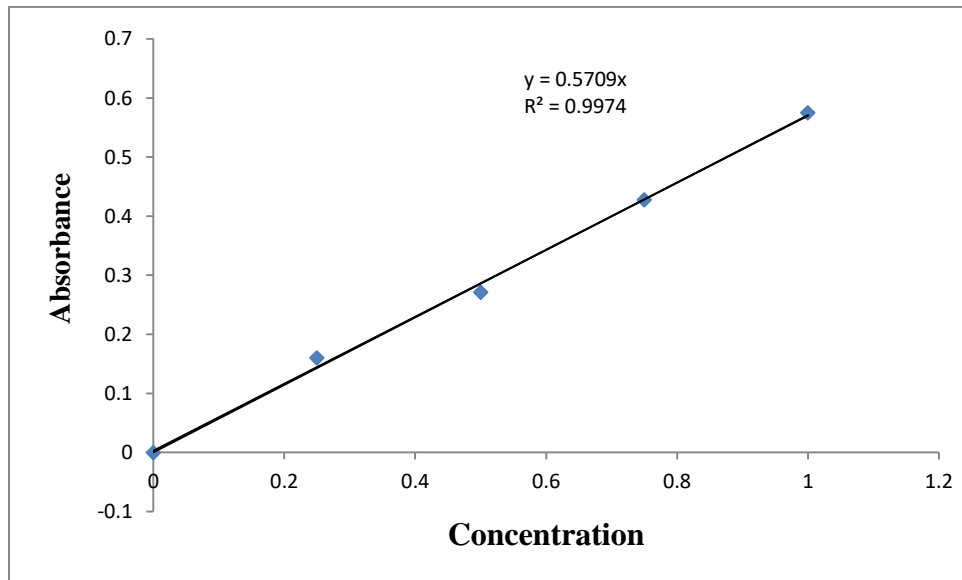


Figure 5. Calibration curve for Zn

(b) Cu

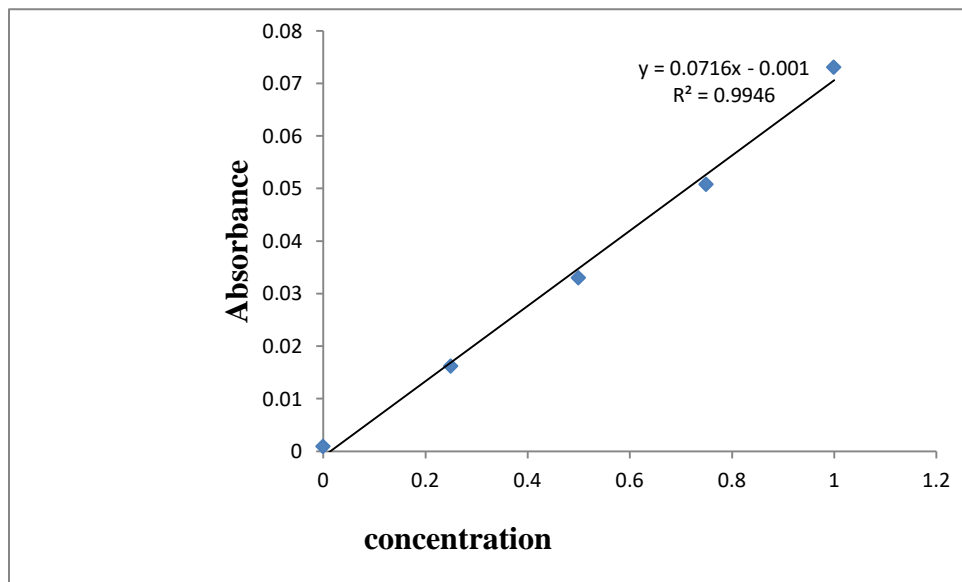


Figure 6. Calibration curve for Cu

(c) Cr

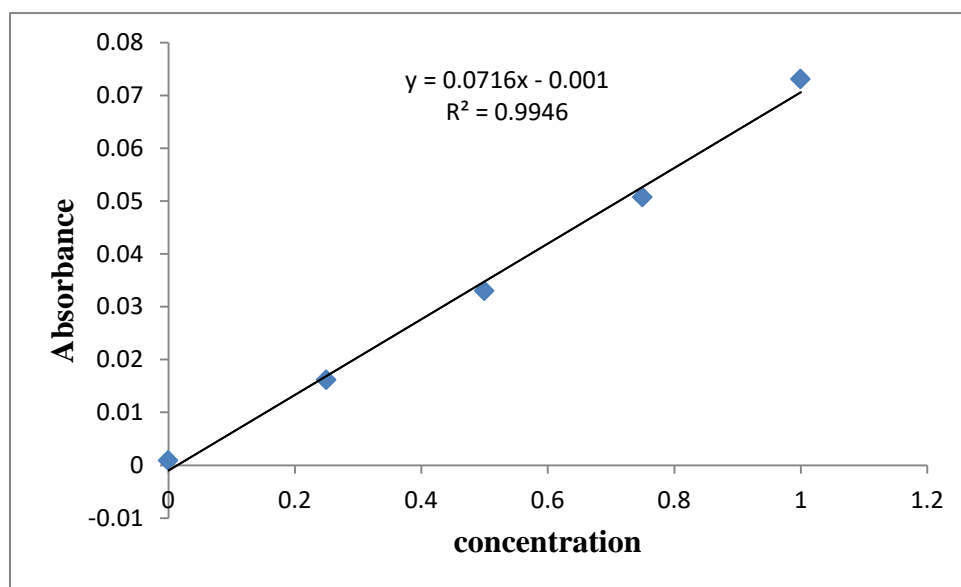


Figure 7. Calibration curve for Cr

(d)

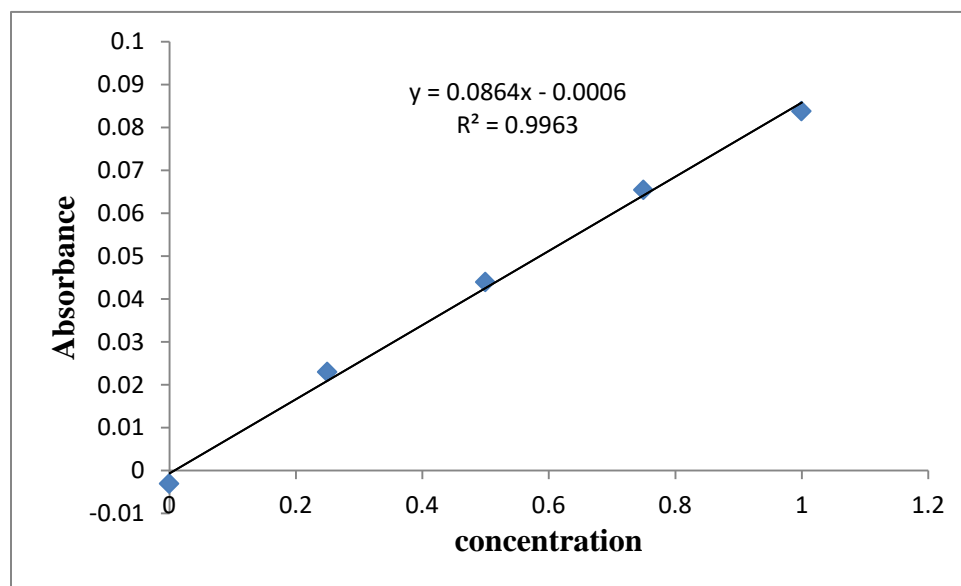


Figure 8. calibration curve for Cd

(e) Pb

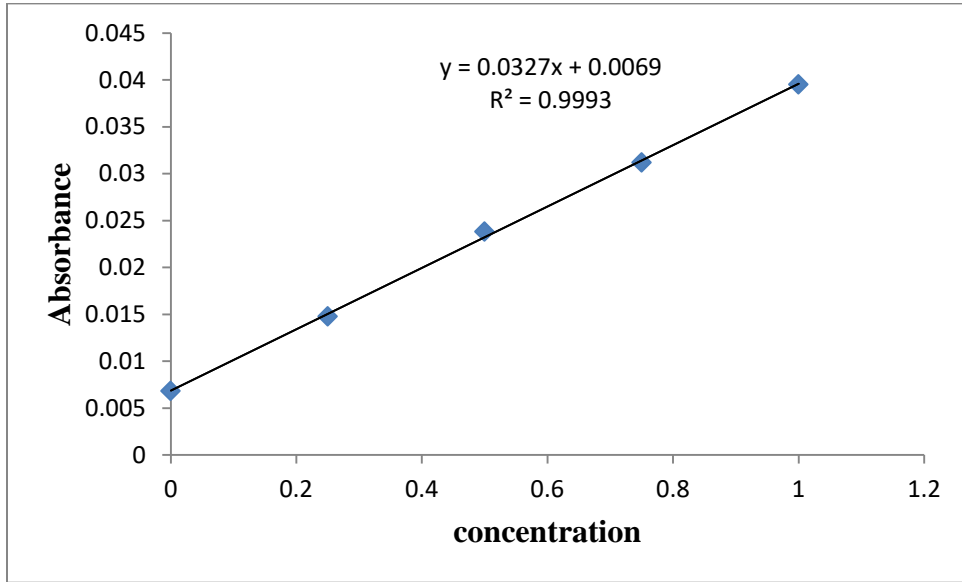


Figure 9. Calibration curve for Pb

The above entire correlation coefficient graphs show that there is strong positive correlation between the concentrations of heavy metals in the samples and the absorbance. The coefficient of determination, R^2 is a statistical measure that tells us how well a regression model fits the data. In other words it tells us how well the model explains the variation in the data. That is it measures the proportion of variation in the dependent variable that can be attributed to the independent variable. The value of R^2 ranges from 0 to 1 and R^2 is 0 means the model doesn't explain any of the variation in the data. A value of 1 means the model explains all of the variation in the data and a value between 0 and 1 means the model explains some of the variation in the data. In this work, all values of R^2 are between 0 and 1 confirming that the model explains the data fitly.

4.2 Method of detection limit and method of quantitation limit

Six blank samples were digested following the same procedure as the samples and each of the samples were analyzed for metal concentrations of Zn, Cr, Cd, Pb and Cu by FAAS. The standard deviations for each element were calculated from the six blank measurements to determine method detection limit and method of quantification of the instrument.

Table 5. Method of detection limit and method of quantification limit of *P. vogelii* and *R. myricoides* samples

Metals	Concentration (mg/L)			
	MDL of <i>P. vogelii</i>	MQL <i>P. vogelii</i>	MDL of <i>R. myricoides</i>	MQL of <i>R. myricoides</i>
Zn	0.0540	0.1601	0.4213	1.400
Pb	0.0502	0.1581	0.0078	0.0262
Cd	0.0070	0.0256	0.0182	0.0597
Cr	0.0130	0.0432	0.0200	0.0654
Cu	0.0057	0.0159	0.0395	0.1309

MDL= method of detection limit and MQL= method of quantification limit

As it can be clearly seen from the above Table MQL is always greater than MDL which confirms that the amount detected is not necessarily quantitated.

4.3 Recovery studies

The spiked samples were digested in triplicate following the same digestion procedure developed previously. The digested spiked samples were finally analyzed for their respective metals using FAAS.

Table 6. Recovery test values for *P. vogelii* samples.

Metals	Concentration (mg/kg)				%Recovery
	Un- spiked	Added	Spiked	Amount recovered	
Zn	75.25	15.050	90.2	14.95	99.3
Cu	17.68	3.622	21.214	3.534	97.5
Cd	3.15	1.22	4.35	1.20	98.4
Pb	10.66	2.34	12.99	2.32	99.1
Cr	BDL	NA	NR	NR	NR

BDL= below detection limit NA = not added and NR = no result

Table 7. Recovery test values in *R. myricoides* leaf samples.

Metals	Concentration (mg/kg)				%Recovery
	Un- spiked	Added	Spiked	Amount recovered	
Zn	10.50	2.100	12.51	2.010	95.71
Cu	34.98	6.996	41.876	6.896	98.57
Cd	4.700	0.94	5.59	0.89	94.7
Pb	18.62	3.724	22.324	3.704	99.4
Cr	BDL	NA	NR	NR	NR

BDL= below detection limit, NA = not added and NR = no result

The percent recoveries of metals in the spiked samples are between 97.5-99.3% in *P. vogelii* and 94.7-99.94 for *R. myricoides*, which confirms that the digestion process totally removed all volatile organic matter leaving behind the inorganic ones and therefore these recovery values are acceptable.

4.4 Evaluation of analytical results

4.4.1 Levels of heavy metals

The concentrations of five heavy metals in both *Peponium voglii* and *Rothecca myricoides* ' sample solutions were determined and identified by FAAS. The levels of metals in *Peponium voglii* with their standard deviations are given in Table 8 and those in *Rothecca myricoides* are given in Table 9

Table 8. Mean concentration, standard deviation and relative standard deviation of each metal in *Peponium voglii*

Metal	Mean(mg/Kg)	Standard deviation	Mean±SD	%RSD
Zn	75.25	±0.038	75.25±0.038	5.1
Cu	17.68	±0.023	17.68±0.023	2.3
Cr	BDL	-----	-----	
Cd	3.15	±0.0066	3.15±0.0066	10.4
Pb	10.66	±0.0019	10.66±0.0019	2.9

Table 9. Mean concentration, standard deviation and relative standard deviation of each metal in *Rothecca myricoides*

Metal	Mean(mg/Kg)	Standard deviation	Mean±SD	%RSD
Zn	10.5	0.0098	10.50±0.0098	9.3
Cu	34.98	0.0046	34.98±0.0046	1.3
Cr	BDL	-----	-----	-----
Cd	4.70	0.0063	8.70±0.0063	7.2
Pb	18.62	0.0072	18.60±0.0072	3.8

The obtained results show that from the five analyzed heavy metals in *Peponium vogelii*, Zn was found to be contained in excess amount and Cr in the least amount; whereas in *Rotheca myricoides* Cu was found to be contained in excess amount and again Cr was found in the least amount which is below detection limit. This difference in the concentration of the same metal in different medicinal plants of the same area is not only due to the difference in the plant types; even in the same plant, different parts accumulate the same metal in different proportions.

In *Peponium vogelii*, the amount of the analyzed metals when arranged in the increasing order is Cr<Cd<Pb<Cu<Zn and in *Rotheca myricoides* the amount of metals in the increasing order is Cr<Cd<Zn<Pb<Cu. In both of these medicinal plants, the most toxic heavy metals Cd and Pb were found; and Pb was found to be the second most abundant metal in while Cd was the second least abundant metal in *Peponium vogelii*. In *rotheca myricoides*, the concentration of Pb was found to be the highest and that of Cd was the second least concentration next to Cr. When the concentrations of the heavy metals in *Peponium vogelii* and *Rotheca myricoides* medicinal plants are represented graphically, the graphs given in following figures were obtained.

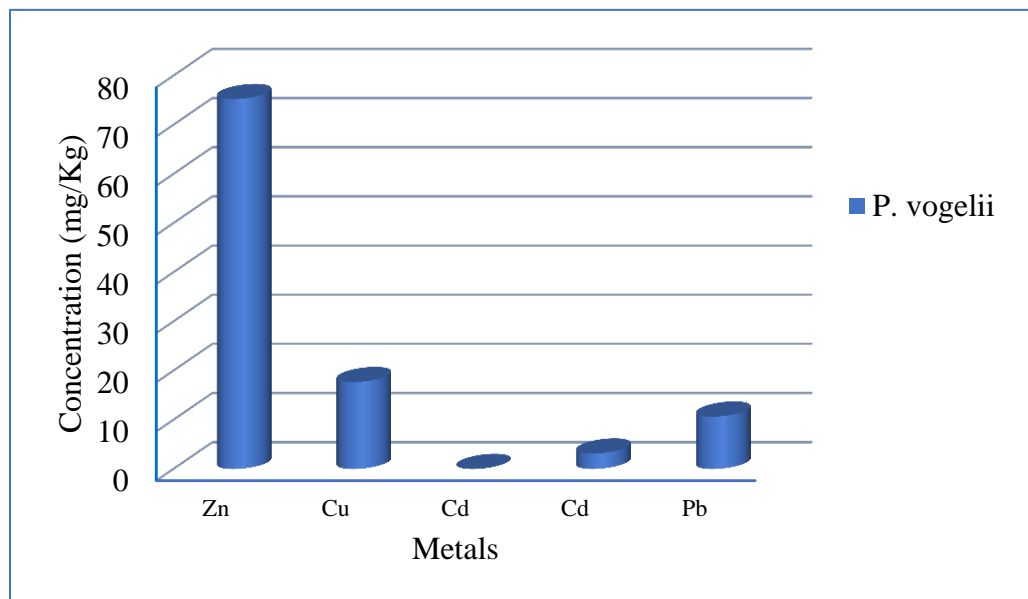


Figure 10. Relative concentrations of heavy metals in *Peponium vogelii*

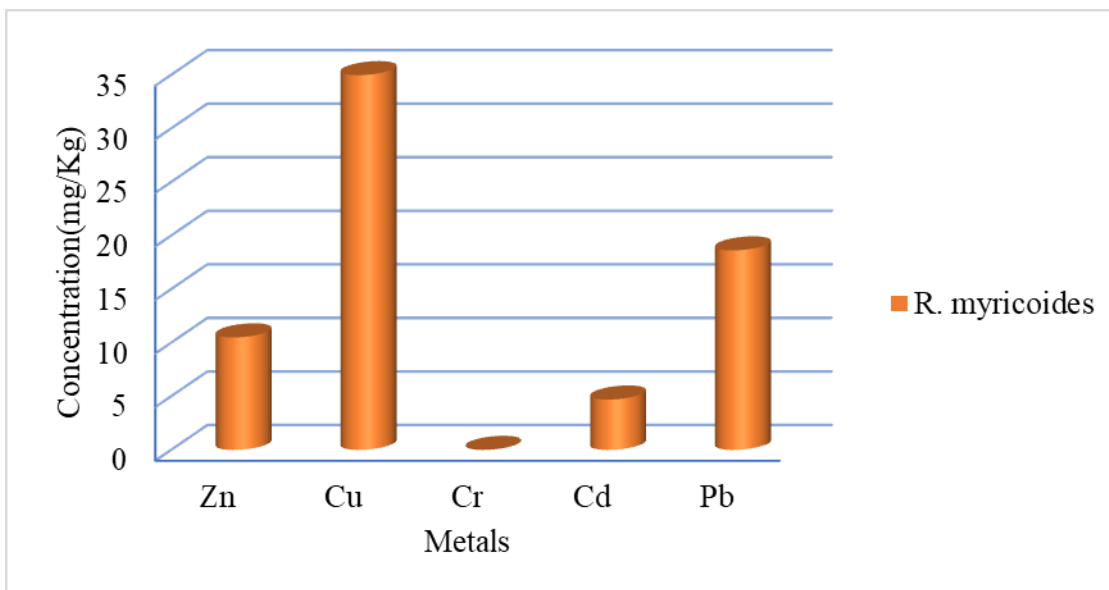


Figure 11. Relative concentrations of heavy metals in *Rotheca myricoides*

As it is clearly seen in table 10 below, the concentrations of all heavy metals except Cr in *Peponium vogelii* and the concentration of two metals in *Rotheca myricoides* with the exception of Zn, are above FAO's/WHO's permissible limit. Therefore, if these medicinal plants are taken frequently and haphazardly, it is obvious that they may cause health problems due to the high concentrations of these metals.

Table 10. The comparison of heavy metals in *Peponium vogelii* and *Rothea myricoides* with FAO's/WHO'S permissible limits (concentrations are in ppm)

Metal	Peponium (mg/kg)	Rothea (mg/kg)	Permissible limits (mg/kg)
Zn	75.23	10.50	27.4-50
Cu	17.68	34.98	2-5
Cr	BDL	BDL	0.02
Cd	3.15	4.70	0.30
Pb	10.66	18.62	5-10

4.5 Comparison of metals in *Peponium vogelii* and *Rothea myricoides*

Even though these two plants are collected from the same area and the same environmental condition, the concentration of heavy metals contained within their body is different. Most probably this could be due to their biological nature of absorbing mineral nutrients from the soil. That is, different types of medicinal plants from the same area and soil may contain the same type of metals in different concentration levels. It is because of this accumulation of toxic heavy metals that some medicinal plants are harmful than others.

As it was given in tables 8 and 9, the concentrations of highly toxic metals are above permissible limits which cause hazardous effects to health. That is according to WHO, the permissible limit for Pb is 5-10 ppm and for Cd it is 0.30 ppm while the concentrations of Pb in *Peponium vogelii* and *Rothea myricoides* are 10.66 ± 0.0019 ppm and 18.62 ± 0.0019 ppm; and the concentrations of Cd respectively are 3.15 ± 0.0066 ppm and 4.70 ± 0.00636 ppm. The following figure illustrates the relative abundance of heavy metals in the two medicinal plants.

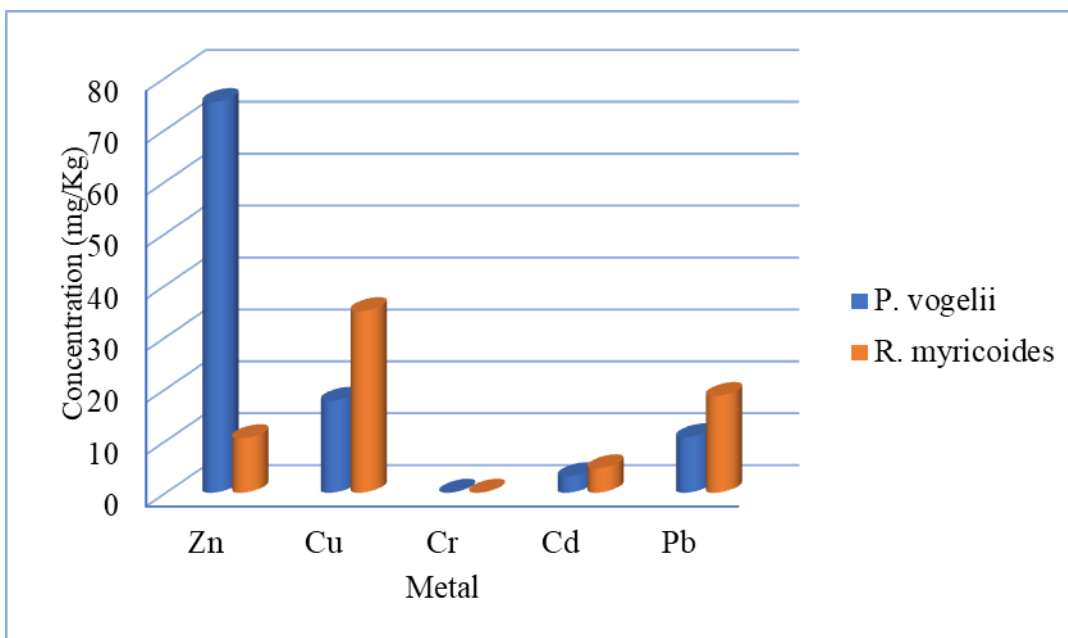


Figure 12. Relative concentrations of heavy metals in *Peponium vogelii* and *Rothea myricoides*

4.5.1 Comparison of the concentration of heavy metals in *Peponium vogelii* and *Rothea myricoides* with those in other medicinal plants

Since traditional herbal medicines are widely used all over the world, many researches have been conducted on them to investigate their side effects. Among those researches, the determination of heavy metals in traditional herbal medicinal plants is the one which has been frequently conducted by many researchers. In a research which has been recently conducted by two researchers in Dilla and Wolayta Universities on three Ethiopian traditional medicinal plants called *Ximenia Americana*, *Withania Samnifera* and *Ziziphus spina Chrishi*, in Amhara region, the concentration of four heavy metals viz. Zn, Cu, Pb and Ni in mg/kg was reported to be as given in the following Table.

Table 11. Comparison of heavy metals in *Peponium vogelii* and *Rotheca myricoides* with those in other Ethiopian medicinal plants (concentrations are in mg/kg)

	Concentrations (mg/Kg) of metals					
	Zn	Cu	Cr	Cd	Pb	Reference
ximenda	14.40	4.50	NS	NS	55.2	[73]
withania	21.20	7.70	NS	NS	4.60	[73]
ziziphus	17.20	3.00	NS	NS	2.00	[73]
P.vogelii	75.25	17.68	BDL	3.15	10.66	This study
R.myricoides	10.50	34.98	BDL	4.70	18.62	This study
Normal	50.00	20.00	1.3	0.2	2.00	-----
Toxic	200.00	20.00	-----	-----	20.00	-----

NB: BDL means below detection limit, NS means not searched and the dashed lines indicate the metals for which regulatory limits have not yet been established by FAO/WHO.

For the metals for which no regulatory limits have been established, their amounts greater than their permissible limits are assumed to be hazardous. The concentration of metals obtained in this work could also be compared with the findings of other researchers reported from abroad. A lot of different papers have been published around the world on the determination of heavy metals in traditional medicinal plants. For example, in one paper published in Iranian journal of toxicology, the maximum concentrations of Cd and Pb obtained from traditional medicinal plants respectively were 1.753 ± 0.0031 and 0.52741 ± 0.003 mg/kg.[74] In another paper published in the journal of environmental and public health by Hindawi Publishing Corporation, the maximum concentrations of the most toxic heavy metals, Cd and Pb reported respectively were 1.11 ± 0.07 and 23.52 ± 1.68 mg/kg.[75] These maximum concentrations are higher than the regulatory limits of FAO/WHO and therefore are believed to cause health problems. This confirms that the casual use of traditional medicinal plants is the worldwide problem to cause health risks to people.

5. CONCLUSION

In this study heavy metals; Zn, Cu, Cr, Cd and Pb concentrations were determined using FAA spectroscopy in two traditional medicinal plants; *Peponium vogelii* and *Rothea myricoides* in Masincho area of Sidama region in Ethiopia. The results of this investigation have showed that the concentrations of Cr in both medicinal plants are below detection limit. The rest four metals in *peponium vogelii*; and the rest two metals except Zn, in *Rothea myricoides* have concentrations above permissible limits. It is obvious that these medicinal plants are certainly believed to cause hazardous effects on the health of the people who frequently consume them as traditional medicines. Particularly the effects due to the presence of the two deadly toxic metals, Cd and Pb needs special attention. Although other heavy metals in the medicinal plants have their own side effects, the presence of the two carcinogenic metals, Cd and Pb above their permissible limits overweighs their hazardous effects to their benefits.

6. RECOMMENDATION

All heavy metals, even the essential metals such as Zn, Cu, Mn, etc., above their permissible limits, have serious side effects upon the health of the people who regularly consume traditional medicinal plants containing these metals. In rural societies, nothing is known about the hazardous effects of traditional medicinal plants; except their advantages. That is most people in such areas die because of the adverse effects of using these medicinal plants containing heavy metals; but the cause of the death is still believed to be the seriousness of the illness because of which they have been taking traditional herbal medicines. Therefore, this society is recommended to be aware of that traditional medicinal plants have not only advantages but also disadvantages. Thus, if other additional types of diseases appear after the patients have started using these traditional medicines, certainly it could be the adverse effects of these traditional medicines and therefore the patients are advised to quit using these medicinal plants and seek for other alternatives.

In this study, only five heavy metals were investigated in only two medicinal plants; *Peponium vogelii* and *Rothea myricoides*. Other researchers are kindly invited to investigate other toxic and carcinogenic heavy metals such as Hg and As which cause acute and chronic human poisoning not only in these two medicinal plants but also in other medicinal plants of this region.

Cultivating medicinal plants in a soil containing high concentration of heavy metals is not recommended, because this contaminates the medicinal plants with toxic heavy metals. Also irrigating cultivated medicinal plants with water polluted with sewage from industries is not recommendable for it may highly contaminate the medicinal plants with toxic heavy metals. The fertilizers used for cultivating medicinal plants should also be tested for the presence of traces of toxic heavy metals.

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