



Phytochemical Composition and Heavy Metals Analysis of Crude Ethanolic Extract of *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale*

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Abstract

Background: The aim of this research was to determine heavy-metal and phytoconstituent contents of *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale*. **Methods:** An atomic absorption spectrophotometer was used to evaluate heavy metals in the ethanolic extract of plant crude powder. Total phenolic and flavonoid contents of the extracts were quantified using standard colorimetric phytochemical assays. **Results:** Iron was detected in all three selected medicinal plants, while copper was detected in *Elaeagnus parvifolia* and *Geranium wallichianum* and was not detected in *Taraxacum officinale*. Available heavy-metal concentrations are summarized in Table 2. Crude extracts of *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale* yielded total phenolic contents of 10.2 ± 0.051 , 34 ± 0.17 , and 5.6 ± 0.028 , whereas the total flavonoid concentrations were 31.31 ± 0.119 , 60.52 ± 0.23 , and 32.10 ± 0.122 , respectively. **Conclusions:** The findings indicate that the selected medicinal plants contain measurable heavy metals and a range of secondary metabolites.

Keyword : Heavy Metals; Phenolics and Flavonoids; Biomonitoring; *Geranium wallichianum*; *Elaeagnus parvifolia*; *Taraxacum officinale*.

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INTRODUCTION

Heavy metal contamination of the environment has become a major global concern because of its wide-ranging negative effects. Expansion of agriculture and metal-processing industries, poor waste-disposal practices, and heavy reliance on fertilizers and pesticides, among other factors, continue to release these inorganic pollutants into rivers, soils, and the wider environment [1]. Among inorganic pollutants, metals such as lead, cadmium, copper, cobalt, and chromium can be harmful to plants, organisms, and humans even at low values. However, heavy metals are not biodegradable and can therefore accumulate in soil, plants, and human vital organs. This situation leads to progressive damage to ecosystems and natural environments. Soils contain trace metals of multiple origins: lithogenic elements are directly inherited from the lithosphere; pedogenic elements are also of lithogenic origin, but their concentration and distribution in soil layers and soil particles are altered due to pedogenic processes; and anthropogenic elements are all those deposited into soils as direct or indirect results of human activities [2].

Medicinal plants are a natural gift for people seeking healthy and invigorating life. Plants have served as a source of both preventive and curative medicine throughout human history, and the use of herbal remedies has grown markedly over the past decade. Traditional medicine also carries deep spiritual significance within tribal communities. The World Health Organization has estimated that a large proportion of traditional medicine practiced in developing countries relies on plant-based extracts [3, 4,

5]. This indicates that herbal medicine plays a vital role in primary health care in developing countries. Herbal ingredients are marketed in various countries around the world to meet the growing demand for herbal therapeutic drugs. Since ancient times, medicinal plants have been used to improve human health and treat various diseases [6].

Phytochemicals fall broadly into two classes: primary and secondary metabolites. Primary metabolites, such as chlorophyll and glucose, are directly required for plant growth, development, and survival. Secondary metabolites, by contrast, are not essential for basic growth or reproduction [7, 8]. Rather, these are low-molecular-weight organic compounds synthesized mainly as a defense against herbivory and microbial or fungal attack, and are not required for core metabolism [8]. They also serve additional roles, including antioxidant activity and protection against UV radiation [9]. Phenolic compounds, alkaloids, cyanogenic glycosides, and terpenes are essential secondary metabolites [10]. Polyphenols are widely studied secondary metabolites with several biological activities, including antioxidant, antibacterial, antimicrobial, anti-inflammatory, cardioprotective, and hepatoprotective effects [11, 12]. It should be noted that plant-derived medicines have historically contributed to the discovery of therapeutic agents [13]. In this study, three medicinal plants, *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale*, were evaluated for their heavy-metal concentrations and phytochemical constituents.

Geranium wallichianum belongs to the family Geraniaceae and genus *Geranium*, and is locally known as Rattenjot. It is a wild herb with a flowering period from June to September. It occurs in the Himalayas from Kashmir to Nepal at elevations ranging from 7,000 to 11,000 feet, and it is also reported in northern regions of Pakistan including Hazara, Azad Kashmir, and the Murree Hills [14, 15]. The plant leaves and components are traditionally used to purify blood and to treat eye, jaundice, and renal problems [16]. Chemical constituents and antioxidant activity have been reported for *Geranium wallichianum* [17], and related studies on *Geranium* species have reported phenolic and flavonoid markers with antioxidant and bioactive properties [18]. Antimicrobial, antifungal, cytotoxic, enzyme-inhibitory, and pesticidal activities have also been documented for *Geranium wallichianum* [19].

Elaeagnus parvifolia belongs to the family Elaeagnaceae and genus *Elaeagnus*, and is locally known as Kankoli. This wild woody shrub flowers between May and August and forms part of the native flora of Nepal, Afghanistan, the Himalayan belt from Kashmir to Bhutan, Assam, and China. Several species within the genus, including *E. parvifolia*, are used in folk medicine and recognized as valuable natural sources of steroids, flavonoids, and triterpenoids. Fruits and seeds of *E. parvifolia* have been reported in traditional use for cough, bronchitis, and pulmonary conditions [20, 21]. People of Azad Kashmir, Pakistan, also use the plant in traditional food and medicinal practices [21, 22]. Although clinical trials on *E. parvifolia* remain limited, pharmacological records for related *Elaeagnus* species have been reported [23].

Taraxacum officinale belongs to the family Asteraceae and genus *Taraxacum*. Dandelion has been reported in traditional and herbal medicine for liver-related and digestive uses [24, 25]. Experimental studies have also evaluated hydroalcoholic extracts and hepatoprotective activity of *Taraxacum officinale* [26, 27]. Recent studies suggest that *Taraxacum officinale* extracts may reduce growth and invasion of selected cancer cell lines [28]. The hepatoprotective effect of *T. officinale* extract has also been studied in animal models of liver injury [29, 30]. The present study was therefore designed to assess the heavy-metal and phytoconstituent profiles of three plant species: *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale*.

MATERIALS AND METHODS

Study area and sampling

This study was carried out in Rawalakot, Poonch District, Azad Jammu and Kashmir, Pakistan. Plant samples were collected from the Rawalakot-Poonch area and taxonomically authenticated at the Department of Botany, University of Poonch, Rawalakot, Pakistan. Heavy-metal analysis was performed at the Central Hi-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan.

Methodology

Selected plant parts (flowers, fruits, and roots) of *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale* were collected from the Rawalakot, Poonch District, Azad Kashmir, and identified and taxonomically authenticated by the Department of Botany, University of Poonch, Rawalakot, Pakistan. All selected parts were dried in the shade and powdered using a grinder. For the preparation of extracts, 70% ethanol in water was used as solvent [31]. Selected plant parts used to prepare extracts for the current research work are listed below.

Plant Extract Preparation

Prior to grinding, plant material was shade-dried for 72 hours. After cleaning, it was pulverized into a fine powder using a mechanical grinder and stored in a sealed, airtight container until further use.

Qualitative Analysis

Standard methods were used to investigate alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids in the hydroethanolic extract of *G. wallichianum*, *E. parvifolia*, and *T. officinale* [32, 33].

Heavy metals determination

Trace element concentrations in the medicinal plants under investigation were quantified using an atomic absorption spectrophotometer housed at the Central Hi-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan. The process recommended by the Association of Official Analytical Chemists (AOAC) was followed, and samples were digested using nitric-perchloric acid [34]. In the first phase, a 250 mL beaker was used, and one gram of wet sample and concentrated HNO₃ (10 mL) were added. A hot plate was used to boil the mixture for 30–45 min to oxidize easily oxidizable materials. The liquid was then allowed to cool before being reheated with 70% HClO₄ (5 mL) until thick white vapors formed. After cooling, distilled water (20 mL) was added and the liquid was heated once more to remove odors. After chilling the solution, the filtrate was prepared in a 50 mL volumetric flask using Whatman No. 42 filter paper and a <0.45 µm filter, and the filtrate was diluted to 50 mL with distilled water. In the second phase, after digestion, an atomic absorption spectrometer (AAS) was used to quantify heavy metals (ppm), including iron (Fe), lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), chromium (Cr), nickel (Ni), cobalt (Co), magnesium (Mg), and manganese (Mn), using the Colagar et al. methodology [35].

Statistical Analysis

For the evaluation of the determined concentration of toxic metals, a descriptive statistical data analysis was applied. Group differences were further assessed statistically using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

This study showed that *E. parvifolia* comprises alkaloids, flavonoids, coumarins, phenols, diterpenes, and starch, while *G. wallichianum* contains alkaloids, flavonoids, coumarins, phenols, glycosides, terpenoids, phytosterols, proteins, diterpenes, and starch. *T. officinale* contains phytochemicals such as alkaloids, saponins, flavonoids, tannins, steroids, carbohydrates, coumarins, glycosides, phenols, and phytosterols, as shown in Table 1. These qualitative findings are consistent with previous phytochemical screening of the same three traditional medicinal plants [36].

Table 1. The results of qualitative phytochemical analysis of hydroalcoholic extract of selected medicinal plants.

Phytochemical class	<i>E. parvifolia</i>	<i>G. wallichianum</i>	<i>T. officinale</i>
Alkaloids	+	+	+
Flavonoids	+	+	+
Coumarins	+	+	+
Saponins	-	-	+
Tannins	-	-	+
Phenols	+	+	+
Glycosides	-	+	+
Phytosterols	-	+	+
Diterpenes	+	+	-
Terpenoids	-	+	-
Starch	+	+	-

Metal Contents of Selected Medicinal Plants

Elements that make up macromolecules, such as C, H, O, and N, were included in Group I. Group II covers minerals essential to nutrition, often termed major elements, which the body requires in amounts exceeding 100 mg per day; a deficiency of these elements can lead to disease. This group includes Na, K, Cl, Ca, P, Mg, and S. Group III elements, also known as trace elements, have a daily requirement of less than 100 mg, but they are essential elements that can cause serious illnesses if deficient. This group includes Cr, Co, Cu, I, Fe, Mn, Mo, Se, and Zn. Cd, Ni, Si, Sn, and Zn are examples of Group IV elements, which are additional trace elements whose role is unknown but possibly essential. Group V elements are toxic to the body and can enter through contaminated air, soil, water, or food substances, such as As, CN⁻, and Hg [37]. The content of metals, essential and toxic elements, such as Cu, Fe, Cd, Pb, Mg, Co, Zn, Ni, and Mn, was investigated in all selected medicinal plants in the current study. In the first phase, samples were digested using AOAC's nitric-perchloric acid method [34]. Mineral contents (ppm) were determined using an atomic absorption spectrometer (AAS) after digestion [35].

Table 2. Concentration of heavy metals in selected medicinal plants (ppm; NR = not reported in the supplied dataset; ND = not detected).

Elements	<i>E. parvifolia</i>	<i>T. officinale</i>	<i>G. wallichianum</i>
Iron (Fe)	768.051 ± 11.53	1065.575 ± 26.58	591.249 ± 8.80
Lead (Pb)	NR	NR	NR
Copper (Cu)	55.268 ± 2.17	ND	203.123 ± 4.98
Cadmium (Cd)	NR	NR	NR
Zinc (Zn)	NR	NR	NR
Chromium (Cr)	NR	NR	NR
Nickel (Ni)	NR	NR	NR
Cobalt (Co)	NR	NR	NR
Magnesium (Mg)	NR	NR	NR
Manganese (Mn)	NR	NR	NR

All tested medicinal plants contained measurable iron (Fe), as listed in Table 2. Copper (Cu) concentrations ranged from 55.268 ± 2.17 ppm in *E. parvifolia* to 203.123 ± 4.98 ppm in *G. wallichianum*, while Cu was not detected in *T. officinale*. Iron (Fe) was highest in *T. officinale* (1065.575 ± 26.58 ppm), followed by *E. parvifolia* (768.051 ± 11.53 ppm) and *G. wallichianum* (591.249 ± 8.80 ppm). Values for Pb, Cd, Zn, Cr, Ni, Co, Mg, and Mn were not reported in the supplied dataset; therefore, these values should be inserted after laboratory confirmation before final publication.

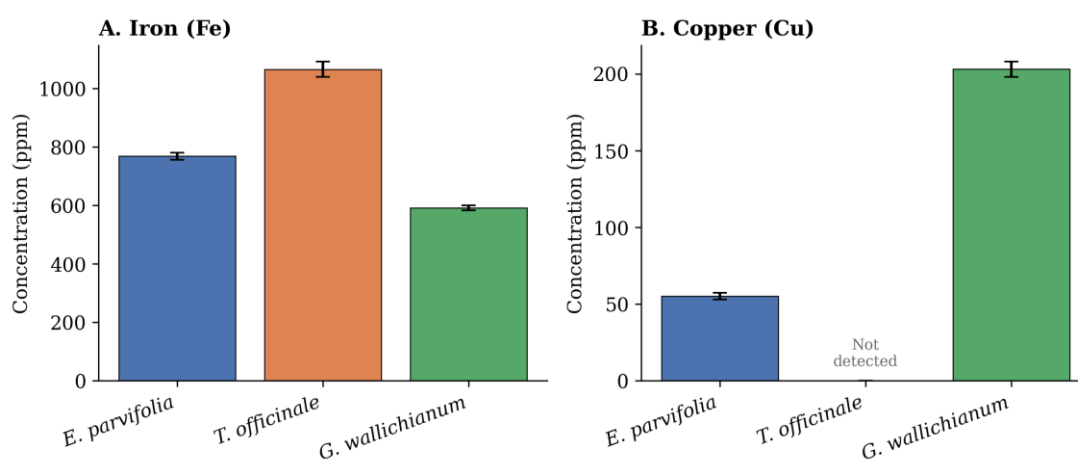


Figure 1. Graphical Representation of Various Heavy Metals' Concentration in Selected Medicinal Plants

The choice of plants was based on the traditional belief of the study area that plant parts are effective in treating various diseases such as diabetes, jaundice, kidney-related problems, eye diseases, and digestive problems. Heavy metals, including lead, cadmium, chromium, and manganese, which can be found in soil and wastewater used for irrigation, can accumulate in these systems. Due to their non-biodegradable nature, the toxic effects of certain metals and their bioavailability in humans can be dangerous. Heavy metals and their interactions with key nutrients can lead to major health issues [38]. The WHO/FAO recommends monitoring toxic metals in foods and herbal materials; Pb and Cd levels in particular warrant close monitoring [39]. Before any chemical action, extraction is an important step in the isolation of plant phenolics and flavonoids [40]. Methanol and ethanol have been shown to be effective extraction solvents, while ethanol is commonly used for plant extraction [41].

CONCLUSION

The results obtained from this study show that the distribution of detected heavy metals and toxic elements in the plant organs of *Taraxacum officinale* is not homogeneous, because it depends on physicochemical parameters and the bioavailability of each element, including specific uptake processes and transfer in organ tissues. The available data show measurable Fe in *Elaeagnus parvifolia*, *Taraxacum officinale*, and *Geranium wallichianum*, and measurable Cu in *Elaeagnus parvifolia* and *Geranium wallichianum*. Cu was not detected in *Taraxacum officinale*. Values for other elements should be inserted after laboratory confirmation before final publication. In addition, a range of phytoconstituents was detected across the plant parts examined.

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التركيب الكيميائي النباتي وتحليل المعادن الثقيلة في المستخلص الإيثانولي الخام لنباتات إبرة الراعي والزيتون البري ، والطرخشقون الملخص

الخلفية: هدفت هذه الدراسة إلى تحديد محتوى المعادن الثقيلة والمكونات النباتية في نباتات إبرة الراعي، والزيتون البري، والطرخشقون. الطرق: استُخدم مطياف الامتصاص الذري لتقييم المعادن الثقيلة في المستخلص الإيثانولي لمسحوق النباتات الخام. وتم قياس محتوى الفينولات والفلافونويدات الكلية في المستخلصات باستخدام فحوصات كيميائية نباتية لونية قياسية. النتائج: تم الكشف عن الحديد في جميع النباتات الطبية الثلاثة المختارة، بينما تم الكشف عن النحاس في الزيت البري وإبرة الراعي، ولم يتم الكشف عنه في الطرخشقون. يُلخص الجدول 2 تركيزات المعادن الثقيلة المتاحة. وقد أظهرت المستخلصات الخام لنباتات إبرة الراعي (*Geranium wallichianum*) ، والزيتون البري (*Elaeagnus parvifolia*)، والطرخشقون (*Taraxacum officinale*) محتوىً إجماليًا من المركبات الفينولية بلغ 0.051 ± 10.2 ، و 0.17 ± 34 ، و 0.028 ± 5.6 على التوالي، بينما بلغ إجمالي تركيزات الفلافونويدات 0.119 ± 31.31 ، و 60.52 ± 0.23 ، و 0.122 ± 32.10 على التوالي. الاستنتاجات: تشير النتائج إلى أن النباتات الطبية المختارة تحتوي على كميات قابلة للقياس من المعادن الثقيلة ومجموعة من المستقلبات الثانوية.

الكلمات المفتاحية: المعادن الثقيلة؛ المركبات الفينولية والفلافونويدات؛ الرصد البيولوجي؛ إبرة الراعي؛ الزيتون البري؛ الطرخشقون.