

PHYTOCHEMICAL COMPOSITION, HEAVY METALS ANALYSIS AND CYTOTOXICITY BY DNA PREVENTION MEASUREMENT OF CRUDE ETHANOLIC EXTRACT OF *GERANIUM WALLICHIANUM*, *ELAEAGNUS PARVIFOLIA*, AND *TARAXACUM OFFICINALE*

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Abstract

Heavy metal toxicity has proven to be a major threat and there are several health risks associated with it. The toxic effects of these metals, even though they do not have any biological role, remain present in some or the other form harmful for the human body and its proper functioning. They sometimes act as a pseudo element of the body while at certain times they may even interfere with metabolic processes. Metal toxicity depends upon the absorbed dose, the route of exposure and duration of exposure, i.e. acute or chronic. This can lead to various disorders and can also result in excessive damage due to oxidative stress induced by free radical formation. Contamination of pharmaceutical products or drug formulations with these toxic heavy metals can compromise patient safety and efficacy. Therefore, stringent quality control measures are essential in drug manufacturing to ensure compliance with regulatory standards.

In this study *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale* was investigated for heavy metals, cytotoxicity and qualitative phytochemical analysis. Atomic absorption spectrophotometer was used to evaluate heavy metals in ethanolic extract of plants. Qualitative phytochemical analysis of the plant extracts was carried out by using standard test. DNA prevention test was carried out to elaborate the toxicity of selected medicinal plants. Results showed that medicinal plants contain phytochemicals including alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids. All these plants contain iron, Zinc, nickel and cobalt in limited concentration that are nontoxic for human body and copper, cadmium and Mercury was not found in any plant while lead was found in small quantity only in *G. wallichianum*. DNA prevention test showed that all selected medicinal plants were nontoxic and showed significant DNA prevention. All these plants are safe for human health and can be used for further investigation for drug development.

Key words: Heavy metals; Phenolic; Flavonoids; Biomonitoring, DNA prevention.

Introduction

All organisms that live in a contaminated environment are influenced by global or local environmental pollution to a certain extent. Among inorganic pollutants, several metals such as lead, cadmium, copper, cobalt and chromium can be harmful to plants, organisms and humans even at quite low values. The heavy metals have the potential to accumulate in soil, plants, and vital human organs as they are not biodegradable. This situation leads to progressive harm effects on ecosystems and natural environments. The soils contain trace metals of many origins: lithogenic elements are directly inherited from the lithosphere; pedogenic elements are of lithogenic origin also, but their concentration and distribution in soil layers and soil particles are changed due to pedogenic processes; and anthropogenic elements are all those deposited into soils as direct or indirect results of man's activities (Giacomino *et al.*, 2016). Air pollution has negatively affected the growth and quality of some plants. Therefore, many researchers in the modern era have called for the possibility of using plants as vital indicators of air pollution. As air pollutants directly affect a plant, which is represented in the accumulation of heavy metals in plant tissues. The accumulation of these heavy metals has a negative impact on human health, causing many diseases (Manisalidis *et al.*, 2020).

Primary components and secondary metabolites are the two major groups of phytochemicals. The chemical components in plant growth and production are the key metabolites. Glucose and chlorophyll, essential for life, are examples of primary metabolites. Secondary metabolites do not participate directly in plant growth and are not necessary to reproduction (Elhardallou, 2011). For many other reasons, secondary plant-generated metabolites are used. It is about regulation of growth, intra and interaction and protection from infections and predators. Phenolic compounds, alkaloids, cyanogenic glycoside and terpenes are some essential side metabolites (Taid *et al.*, 2014). Polyphenols are the most used secondary metabolites that have a wide range of biological activities including antibacterial, anticarcinogenic, antiviral, hepatoprotective, cardioprotective, antithrombotic, anti-allergic, anti-inflammatory and infertility treatment (Lia *et al.*, 2008; Trigui *et al.*, 2013). It is noteworthy that plant derived medicines are often used to cure of various diseases by humans (Sharma *et al.*, 2009).

In this study three medicinal plants *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale* were evaluated for heavy metals concentration of important trace elements. These plants are distinguished from others in that they contain chemical compounds used in the elimination of insects, treatment of diabetes, high blood pressure, arteriosclerosis, anti-

fungal and anti-bacterial, and anti-cancer. The richness of these plants in chemical compounds may reduce the medical effectiveness due to the lack of research that is based on it to evaluate its effectiveness compared to manufactured chemical treatments (Uritu *et al.*, 2018). The use of herbal medicines has increased dramatically over the last decade. The World Health Organization (2007) estimates that 80% of traditional medicine in developing countries is for healthcare and includes herbal extracts (Prasad *et al.*, 2014). This indicates that herbal medicine plays a vital role in primary health in developing countries. Herbal ingredients are marketed in various countries worldwide to meet the growing demand for herbal therapeutic medicines. From antique times, herbal medicines have been used to improve human health and treat various diseases (Pandey, 2014).

Geranium wallichianum belongs to family Geraniaceae and Genus *Geranium* locally known as Rattenjot. It is a perennial plant that grows to approximately 0.3 m (1ft) by 1.5 m (5ft in). It is a hermaphrodite plant that is pollinated by insects. It bears fruit from July to September. It may be found in the Himalayas from Kashmir to Nepal, at elevations ranging from 7000 to 11000 feet (Fig. 1).

It could also be found in the Northern parts of Pakistan, including Hazara, Azad Kashmir, and the Murree Hills (Ahmad *et al.*, 2003). The plant leaves and components are used to purify the blood, treat eye, jaundice and renal problems (Qureshi *et al.*, 2007). Hepatitis, prolonged fever and leukorrhea are also treated with this genus (Adeel *et al.*, 2011). Six compounds (sitosterol, ursolic alkaline, stigmasterol, galactoside, hernal and hydroxyethyl benzoate) are believed to have cell-enhancing activity of cellular ethyl acid derivatization at a powerful IC 19.05 g/ml for n-butanol. and high-water concentrations (Ismail *et al.*, 2009). Targeted studies have shown that another species, *Geranium* has phytochemical markers for phenolic compounds, particularly flavonoids, in terms of their strength and cellular compatibility and hyperglycemic effects (Vania *et al.*, 2016). Antimicrobial, antifungal, cytotoxic, protein inhibitor, and pesticidal activity have been documented as well (Ismail *et al.*, 2012).

Elaeagnus parvifolia belongs to Family Elaeagnaceae and Genus *Elaeagnus* locally known as Kankoli. It is a vigorous tree with abundant cream-colored flowers, blooming from April to May. It is a medium-tall shrub 4.5 m (14ft) by 3 m (9ft) at a medium rate. It is a significant part of Nepal's flora, as well as Afghanistan, the Himalaya (from Kashmir to Bhutan), Assam, and China. Several species of *Elaeagnus*, notably *E. parvifolia*, have been isolated of steroids, flavonoids, and triterpenoids, and are regarded folk medicinal plants. Cough and bronchitis have been reported to be cured by their fruits (Pant, 2010; Singh *et al.*, 2014). The fruit is used to fight against poor growth. Impregnation is used as a diuretic. People of Azad Kashmir, Pakistan used it as a traditional jam and stick forming practice (Khan *et al.*, 2010). It is used to treat respiratory and penetrating diseases of patients in Azad Kashmir, Pakistan (Amjad *et al.*, 2015). According to textual research, there is no evidence of clinical trials on *E. parvifolia*. However, clinical records for different *Elaeagnus* species are recorded (Liao *et al.*, 2012) (Fig. 2).

Taraxacum officinale belongs to the family Asteraceae and Genus *Taraxacum*. In traditional Chinese medicine combines dandelion with various spices to treat hepatitis (Blumenthal *et al.*, 2000; Modaresi *et al.*, 2012; Mahesh *et al.*, 2010). It is also used in the treatment of jaundice, poisoning, blood clots, fever, eye, digestive problems, osteoporosis, dermatitis and malignant cancer of the uterus and breast in women (Modarsiet *et al.*, 2012). Recent studies suggest that it may reduce the risk of travel disorders, including growth (Sigstedt *et al.*, 2008). The hepatoprotective effect of *T. officinale* fluid is under study and has been found beneficial for the management of hepatitis B (Park *et al.*, 2007). It has also been found to protect effects against severe liver damage caused by carbon tetrachloride in mice (Park *et al.*, 2010).

Taraxacum officinale is one of the most widely used plants as an indicator of heavy pollution due to its large spread, ease of access and low cost, in addition to the large accumulation of heavy metals in its tissues, which facilitates its use as an atmospheric monitor for air pollution (Fig. 3).

This study aims to measure the amount of iron, lead, copper, cadmium, zinc, chromium, nickel and cobalt in different concentrations of solutions extracted from *Geranium wallichianum*, *Elaeagnus parvifolia*, and *Taraxacum officinale*, in addition to the detection of secondary metabolites in those plants.

Material and Methods

Study area and sampling: This study was performed in the University of Poonch Rawalakot Azad Kashmir.

Methodology: The selected plant *Geranium wallichianum*, *Elaeagnus parvifolia* and *Taraxacum officinale* were collected from the Rawalakot Poonch district, Azad Kashmir and identified and authenticated taxonomically from Department of Botany, University of Poonch, Rawalakot, Pakistan. All the selected parts were dried under shade, powdered using grinder and for the preparation of extracts 70% ethanol in water was used as solvent. The parts of selected plants used to prepare extracts for current research work are given below.

Plant extract preparation: The plant materials were dried in a shade for 72 hours the dried plant parts may have like 12–20% moisture and prepared for grinding. After cleaning, the contents of the plants were ground into a fine powder fitted with a stirrer and stored in an airtight container with suitable sealants for future use. (Tchounwou *et al.*, 2012).

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* (Shanmugam *et al.*, 2010, Ayaz *et al.*, 2014).

Heavy metals determination: The content of key trace elements in examined medicinal plants were measured using an atomic absorption spectrophotometer available

at the Central Hi-Tech Laboratory, University of Poonch Rawalakot, Pakistan. The process recommended by the Association of Official Agricultural Chemists (AOAC, 2000) was divided into two parts, with the sample being digested using nitric-perchloric acid. In the 1st phase, 250ml capacity beaker was taken and one gram of wet sample and concentrated HNO₃ (10ml) were taken. Hot plate (Model) was used to boil the mixture to oxidize all easily oxidizable materials for 30-45 min. The liquid was then allowed to cool before being re-heated with 70% HClO₄ (5ml) until thick white vapors formed. After cooling, distilled water (20mL) was added, and the liquid was heated one more time to remove any odours. After chilling the solution, the filtrate was prepared in a 50mL volumetric flask using Whatman No. 42 filter paper and <0.45 mL filter paper, and the filtrate was diluted to 50mL with distilled water. In 2nd phase, after digestion, an atomic absorption spectrometer (AAS) was used to quantify heavy metals ($\mu\text{g g}^{-1}$) including Magnesium (Mg), Zinc (Zn), Iron (Fe), Calcium (Ca), Arsenic (As), Cupper (Cu), Phosphorus (P), Cadmium (Cd), Lead (Pb) and Mercury (Hg) using the Colagar *et al.*, (2009) methodology.



Fig. 1. *Geranium wallichianum* with flowers.



Fig. 2. *Elaeagnus parvifolia* with fruits.



Fig. 3. *Taraxacum officinale* with flower.

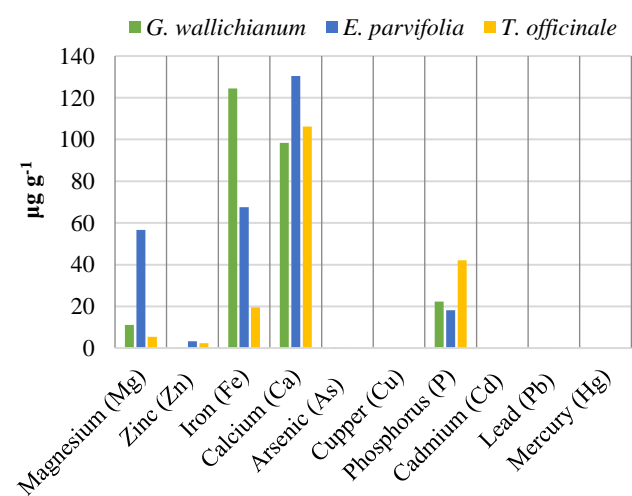


Fig. 4. Graphical representation of concentration of various heavy metals in the selected medicinal plants.

Statistical Analysis

For evaluation and assessment of the determined concentration of toxic metals, a descriptive statistical data analysis was applied. Data analysis was statistically processed by using One-way analysis of variance (ANOVA).

Results and Discussion

Qualitative phytochemicals: This study showed that *E. parvifolia* comprises alkaloid, flavonoid, coumarins, phenols, diterpenes and starch while *G. wallichianum* contain alkaloid, flavonoid, coumarins, phenols, glycosides, terpenoids, phytosterols, proteins, diterpenes and starch. *T. officinale* have phytochemicals like alkaloids, saponins, flavonoids, tannins, steroids, carbohydrates, coumarins, glycosides, phenols and phytosterols as shown in (Table 1).

Table 1. 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: The elements that make up macromolecules, such as C, H, O, and N were included in Group I. Minerals that are nutritionally important and are also known as principal elements which were included in Group II. Malnutrition of this element can cause diseases and it is necessary to consume more than 100mg per day. 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 100mg, but they are essential elements that can cause serious diseases if they are deficient. This group includes Cr, Co, Cu, I, Fe, Ca, Mo, Se, and Zn. Cd,

Ni, Si, Sn, and Zn are examples of Group IV elements, which are additional trace elements whose play role is unknown but are possibly essential. Group V elements are those that are toxic to the body and will enter the body through contaminated air, soil, water, or food substances, such as, CN⁻, and Hg (Chatterjee & Shinde, 2011). Metal content, both essential and toxic elements, such as (Mg), (Zn), (Fe), (Ca), (As), (Cu), (Fe), (Cd), (Pb) and (Hg) were investigated in all selected medicinal plants in the current study.

In the first phase, samples were digested using the AOAC's (2000) nitric-perchloric acid method recommended by the AOAC. Colagar *et al.*, (2009) was used to determine mineral contents ($\mu\text{g g}^{-1}$) using an atomic absorption spectrometer (AAS) after one gram of the wet sample was absorbed (Table 2).

All of the medicinal plants tested contain significant concentrations ($\mu\text{g g}^{-1}$) of the minerals listed in (Table 3). Magnesium (Mg) concentrations ranged from $5.42 \pm 0.43 \mu\text{g g}^{-1}$ to $56.7 \pm 1.6 \mu\text{g g}^{-1}$, with *Elaeagnus parvifolia* having the highest concentration and *Geranium wallichianum* having the lowest concentration. Zinc concentration was highest in *Elaeagnus parvifolia* (3.34 ± 0.41) followed by *Taraxacum officinale* ($2.32 \pm 0.53 \mu\text{g g}^{-1}$) and *Geranium wallichianum* have lowest ($0.176 \pm 0.02 \mu\text{g g}^{-1}$), Iron (Fe) in increasing concentrations ($92.4 \pm 5.1 \mu\text{g g}^{-1}$) was in *Geranium wallichianum* and in lowest concentration was in *T. officinale* ($23.5 \pm 1.24 \mu\text{g g}^{-1}$) while the concentration of (Fe) in *E. Pervifolia* was ($67.5 \pm 3.21 \mu\text{g g}^{-1}$). The highest concentration of Calcium (Ca) was found in *Elaeagnus parvifolia* ($130.41 \pm 0.23 \mu\text{g g}^{-1}$), followed by *Taraxacum officinale* ($106.23 \pm 0.05 \mu\text{g g}^{-1}$), and *Geranium wallichianum* ($98.32 \pm 0.51 \mu\text{g g}^{-1}$) in decreasing order.

Arsenic (As) levels in *Elaeagnus parvifolia* and *Taraxacum officinale* were found in limit describe by WHO but slightly higher in *Geranium wallichianum*. Lead (Pb) is a toxic element, and concentration of Lead (Pb) was found lower from WHO limit. Copper (Cu), Cadmium (Cd) and Mercury (Hg) was non detectable in selected medicinal plants *Taraxacum officinale* and *Geranium wallichianum*. Furthermore, results of Phosphorus (P) levels revealed that *Elaeagnus parvifolia* ($22.3 \pm 2.1 \mu\text{g g}^{-1}$), *Taraxacum officinale* ($42.03 \pm 1.5 \mu\text{g g}^{-1}$) and *Geranium wallichianum* ($18.20 \pm 1.32 \mu\text{g g}^{-1}$) was found (Table 3, Fig. 4).

Table 2. Permissible limits (Anon., 1999) of the heavy metals ($\mu\text{g g}^{-1}$).

Permissible limits	Cadmium	Lead	Arsenic	Mercury
	0.1	0.2	0.1	0.03

Table 3. Concentration of heavy metal in selected medicinal plants.

	<i>G. wallichianum</i>	<i>E. parvifolia</i>	<i>T. officinale</i>
Magnesium (Mg)	11.15 ± 0.56	56.7 ± 1.6	5.42 ± 0.43
Zinc (Zn)	0.176 ± 0.02	3.34 ± 0.41	2.32 ± 0.53
Phosphorus (P)	22.3 ± 2.1	18.20 ± 1.32	42.03 ± 1.5
Calcium (Ca)	98.32 ± 0.51	130.41 ± 0.23	106.23 ± 0.05
Arsenic (As)	0.302 ± 0.13	0.034 ± 0.03	0.091 ± 0.21
Copper (Cu)	ND	ND	ND
Iron (Fe)	92.4 ± 5.1	67.5 ± 3.21	23.5 ± 1.24
Cadmium (Cd)	ND	ND	ND
Lead (Pb)	0.046 ± 0.01	ND	ND
Mercury (Hg)	ND	ND	ND

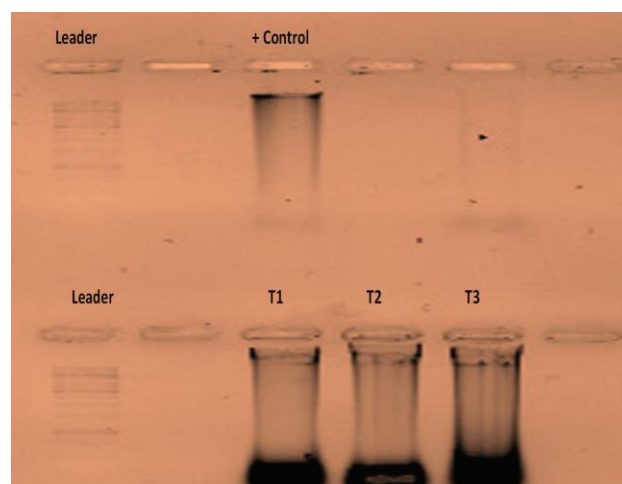
From the given findings, it is concluded that the levels of these elements are within the standard parameters. Our study completely agreed with the results which confirmed that concentration of heavy metals in these medicinal plants were within the toxic limits. Though, its concentration varies based on different environmental conditions. These metals are very necessary for the development of plants, and its excess or shortage can cause serious problems in plants and humans. Zn is an essential trace nutrient for plant growth due to its role in several cell functions. It is also vital for brain growth, normal growth, bone formation, wound-healing, and behavioral response with a dietary limit in humans of 100 ppm (Khan *et al.*, 2019). Cu is important for normal plant growth, but its extreme levels (>100 ppm) can cause phytotoxicity. In these samples, Pb, As, Cd, and Cr were not detected or in very minute amount. Industries, sewage, air, water pollution, and the fly ash are the main sources of heavy metal contamination. These plants were collected from the wild flora of Kashmir. It is away from cities and industries or industries sewage. This may be the reason why these plants are safe.

The plants choice was based on the traditional belief of the study area that, the plant parts are effective in the treatment of various diseases such as diabetes, jaundice, kidney related problems, eye diseases and digestive problems. These systems are susceptible to the buildup of heavy metals like lead, cadmium, chromium, and manganese, which can be found in soil and irrigation waste water. Due to its inanimate nature, the toxic effects of certain plants and therefore their bioavailability in humans can be dangerous. Major health problems can be caused by heavy metals and their interactions with essential nutrients. The WHO advises that metal usage in prefabricated plants be regulated. Pb and Cd are crucial for figuring out which bodily function is useless. Pb poisoning results in seizures, chronic nephritis, problems with the central nervous system, and colic, whereas Cd poisoning damages the liver and kidneys over time when it builds up in the body. The negative effects of extended Cr use include skin rashes, nasal irritation, kidney and liver damage, haemorrhage, inflammation, and lung cancer (Ara *et al.*, 2015).

Prior to any chemical action, extraction is an important step in the exclusion of plants (phenols and flavonoids) (Murugan and Parimelazhagan, 2014). The crude methanol production in our study included in three different plants. Methanol and ethanol have been shown to be effective extraction solvents, while ethanol is the most commonly used solvent for plant extraction (Shi *et al.*, 2004).

DNA damage prevention test: First, 1X TAE buffer was made in the current study's *In vitro* experiment by dissolving 10 ml of 50 X buffer in 490 ml of distilled water. Then, 1 g of agarose gel was dissolved in 100 mL of 1X TAE buffer to create an agarose gel solution that was 1 percent. After that, it was baked in the oven for one minute to create a uniform mixture. This was warmed just enough to a comfortable temperature. Agarose gel received 20 L of the staining ethidium bromide dye. This was mixed and then added to the gel tray of the gel electrophoresis apparatus. This was given 30 minutes to set up. After solidification, 1X TAE buffer was added until both electrodes were submerged in the solution. Each reaction mixture received 3 L of the loading dye bromophenol blue following incubation. Then, these samples were added to the TAE buffer and ethidium bromide-containing wells

that had been created using combs on the 1 percent agarose gel. In a gel electrophoresis device, each reaction mixture with a column was run horizontally in TAE buffer at 100 volts for 45 minutes (Fig. 5).



DNA remain intact in all samples that indicating samples are not cytotoxic.

Fig. 5. DNA damage prevention potential of selected medicinal plants using Ct DNA (Calf thymus DNA).

Some medicinal plants may contain mutagenic molecules, posing a risk of carcinogenic hazards to their users when used as a medication or food supplement for long periods of time. Medicinal plants with genotoxic properties must be declared genetically toxic and labelled as dangerous. As a result, it's critical to identify genotoxic herbs and explain why they should be used with caution (Rashed *et al.*, 2015). Furthermore, determining the antimutagenic activities of medicinal properties is critical in determining the chemopreventive properties of medicinal plants as well as their therapeutic potential for clinical use. Antimutagens compounds prevent mutagenicity by preventing DNA-mutagen interactions (Bhattacharya, 2011). The oxidative damage of DNA is one of the most important mechanisms in the initiation of cancer and this damage is usually caused by OH radicals (Reddy *et al.*, 2005). In this study, the extract displayed considerable protective activity of DNA damage and could, therefore, be safe.

Conclusion

The results of the study indicated that the distribution of heavy metals in the plants models used is not homogeneous due to the physical and chemical properties, biological diversity, and the degree of uptake. The benefit of this study was not to use these plants in these places in the process of traditional herbal treatment because they contain heavy metals that may lead to human life and cause him many health problems.

Elaeagnus parvifolia, *Taraxacum officinale*, and *Geranium wallichianum* all include Magnesium (Mg), Zinc (Zn), Phosphorus (P), Calcium (Ca), Arsenic (As) and Iron (Fe), according to this study. Although all of the plant extracts showed DNA remain intact in all samples that indicate that samples are not cytotoxic.

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