COMPARATIVE STUDY OF METALS AND PHYTOCHEMICAL SCREENING IN AQUEOUS AND ACETONE EXTRACTS OF *CALOTROPIS PROCERA* AND *AJUGA BRACTEOSA*

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Abstract. The main purpose of this study was to evaluate the antioxidant and phytochemical activities and metal contents from leaves, flowers and roots of regional Aak (*Calotropis procera* Ait.) and Hari Boti (*Ajuga Bracteosa* Wall.). Distilled water and acetone solvents were used to extract the antioxidants and phytochemical constituents. Diverse antioxidant and phytochemical activities such as flavonoid, phenolic, and flavonol contents, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), OH°, and H_2O_2 scavenging activities, Fe^{+2} chelating action, ferric reducing antioxidant power (FRAP), ascorbic acid and phosphomolybdenium complex essay were performed by using reported methods. Atomic absorption spectroscopy was used to evaluate the different metals such as Pb, Cd, Zn, Fe, Cu, Mg and K. The flavonoid contents (expressed as rutin equivalents) stated as 12.01-82.56 mg/g dry weight were higher than phenolic contents (expressed as gallic acid equivalents) quantified as 26.60-66.60 mg/g dry weight. *Ajuga bracteosa* extracts exhibited significant DPPH and OH radical scavenging activities. In all the analyzed samples, the highest concentration of iron (27.97 mg/L) was displayed by roots of *Ajuga Bracteosa.* Health risk index (HRI) revealed that level of Cd in roots and flowers of *Calotropis procera* and in flowers of *Ajuga bracteosa* was not safe for the consumption of humans as its concentration exceeded permissible limit.
Keywords: *ascorbic acid, Fe^{+2} chelating action, acetone, ferric reducing antioxidant power*

Introduction

Since old times, people are relying onto medicinal plants for their sicknesses. History for medicinal plants may be longstanding concerning illustration of mankind's historical backdrop. Since the mid of the 19th century diverse categories regarding bioactive compounds have been separated, characterized and identified. Among these, a number of compounds played a prime role in the discovery of new drugs. Many medicines manufactured using plant ingredients possess significant amount of alkaloids, tannins, flavonoids, and phenolic compounds, which play effective role in the cure of a variety of degenerative diseases (Ghias et al., 2012; Ali et al., 2015). Antioxidants help on chain softening responses and free radical searching action as well. All radicals have unpaired electrons and are profoundly breakable, also could extricate electrons starting with other particles to accomplish strength bringing on them harm. An over the top processing about reactive oxygen species (ROS) induces the oxidative stress thereby generating harm to DNA, proteins or lipids, and hampering their normal working (Pham-Huy et al., 2008). These biochemical alterations would involve in the aging process, and additionally in a developing rundown for mankind's diseases, for example, cancerous (Ziech et al., 2011), furthermore Alzheimer's disease (Galindo et al., 2010).

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Cancer and cardiovascular diseases are considered main causes of death in United States of America (USA). Approximately, 32% deaths due to cancer could be evaded by nutritional adaptations. Extracts from curative herbs, vegetables, and fruits possess anticancer activity, akin to hormonal cure and chemotherapy (Wang et al., 2018). Oral doses of *Catharanthus roseus* flowers' aqueous extract to rats (diabetic) caused remarkable decrease in blood glucose, and decline in lipid profile. This oral administration also controlled the drop off in body weight (Suja, 2018). Fruits, vegetables and grains hold numerous widespread mixtures of phytochemicals. Antioxidants inferred from plants go about as diminishing operators, metal molecule chelators and also free radical scavengers. In the coming years, exceptional consideration are required to be paid towards exploring natural-based cell reinforcement extraction starting with plants such as, phenols, flavonoids and tocopherols (Wojdyłoa et al. 2007; Katalinic et al. 2006). Toxic metals accumulated in medicinal plants grown on contaminated soil might represent a danger to human wellbeing. Indeed purpose of ongoing research should generally be to lower the levels of contaminates. In national priorities list (NPL) of the substances which are established to cause the potential threat to human health because of recognized or alleged toxicity, arsenic ranks at first. Agency for toxic substances and disease registry (ATSDR) prioritizes substances in light of a mix about their toxicity, possibility of mankind's purposes of presentation and also recurrence. Lead, mercury, and cadmium rank at 2^{nd}, 3^{rd} and 7^{th} positions in the list of toxicants, separately (ATSDR, 2017). Mankind's interaction with metals might happen throughout world related activities principally through inward breath, dermal routes, mining and from water. Furthermore, contaminated food utilization and exposure to polluted air, soil and dust also enhance metal level in humans (Carlin et al. 2016). A thorough survey from claiming phytochemistry, medicinal and universal utilization of *Calotropis procera* (Aak) and *Ajuga bracteosa* (Kauri Boti) may be distributed by Ahmed et al. and Mubashir Hussain et al. separately (Ahmed et al. 2005; Mubashir Hussain et al. 2016). *Calotropis procera* had been used to treat large portions of infections such as ulcers, spoiling about skin, leprosy, piles, bronchial asthma, and illnesses of the liver, abdomen and spleen as well (Kartikar and Basu, 1994). *Ajuga bracteosa* is over 10-30 cm in stature also a prised, medicinal, soft, fragrant and villous herb. Furthermore, goes about similarly as antibacterial, anti-inflammatory, astringent, anthelmintic and antifungal. It can be used to cure intestinal infections, fever, phlegm, gout, palsy, amenorrhea and stiffness (Shen et al., 1993; Kaithwas et al., 2012). Different metals play a vital role in human body. Metals are characterized into two categories; fundamental or key metals and unnecessary or harmful metals. Key metals are copper, iron and Zinc, whereas nonessential or lethal metals are cadmium, chromium, lead and mercury. Key metals also become toxic when taken in high amount (Angelova et al., 2004). Intake of metals through intake of polluted therapeutic plants is related to human wellbeing dangers (Khan et al., 2007). Appraisals of wellbeing dangers to metals are as per the following; Fe is a basic element which limits many body capacities including supply of O_2 in the blood. Fe is also fundamental for giving vitality to the body. Praline hydrolase, ribonucleotide reductase, pyruvate oxidase, mitochondrial cytochrome, and tyrosine are the vital catalysts that entail the iron as a cofactor in the human body (Manore et al., 2009).

Copper assumes a central part in metabolism of energy, amalgamation and assurance of collagen protein. Proteins that oblige the Copper (II) particle as a co-variable in the body are superoxide dismutase and cytochrome-c-oxidase. Raised convergence of Cu in

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human bodies causes many infections like gastrointestinal infection (Turkdogan et al., 2002). High accumulation of lead has troublesome health impacts such as respiratory and dermatogenic issues brought on by ingestion and dermal contacts of polluted soil (Cao et al., 2010; Wang et al., 2009). Abnormal amounts of lead cause hypertension, stomach related and apprehensive clutters, memory and focus issues, muscle and joint torment. Zinc is a critical mineral required for the body. Zinc controls many body functions, similar to resistant and stomach related framework, lessening of stress levels, vitality digestion and curing of wounds. Deficiency of zinc causes numerous infections such as low circulatory strain, development impediment and hindered bone improvement. Cadmium is not viewed as fundamental to human life. Unfriendly wellbeing impacts on individuals are displayed by overabundance cadmium introduction. From 20 to 30 years, cadmium is aggregated in the kidneys for a moderately extensive period and, at high measurements, harm respiratory framework and causes bone sickness. In the study zone these natural plants have been used to cure skin infections, fever, gout and asthma. The point of the available assessment may be to examine the cell reinforcement exercises, furthermore lethal levels for follow metals, additionally assess supplementary metals starting with chosen parts of *Ajuga bracteosa* and *Calotropis procera* developed in the region Bhimber AJK, Pakistan, eventually Tom's perusing utilizing different extraction solvents.

Methods and materials

Instruments/devices used

Double-beam UV-Visible Spectrophotometer, Model UV-1900, Shimadzo, AA-7000 Atomic Absorption Spectrophotometers, Shimadzo, and diamond saw blades (Cutter Diamond) were used in this study.

Reagents and chemicals

Folin-ciocalteu, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine, hydrogen peroxide, L-ascorbic acid, and Rutin were purchased from Sigma Chemical Co. whereas H_2SO_4, H_3PO_4, Na_3PO_4, $HClO_4$, HNO_3, $(CH_3)_2CO$, Na_2CO_3, $NaNO_2$, $AlCl_3$, NaOH, CH_3COONa,), CH_3OH, $FeSO_4$, $C_6N_6FeK_3$, $C_2HCl_3O_2$, $FeCl_3$, HO_3P, $C_{12}H_7NCl_2O_2$, $(NH_4)_6Mo_7O_{24}$ were obtained from Merck (Germany).

Sampling area

The district Bhimber is situated at an altitude of 313 meters above sea level. It is located between latitude: 32-48 to 33-34 and longitude: 73-55 to 74-45, and has an area of 1516 km². People living in the rural and urban areas of district Bhimbr frequently use extracts of various parts of medicinal plants such as roots, leaves and flowers to cure ailments. Hence, the district was focused for this study (*Fig. 1*).

Sampling of diverse parts of plants

The study was aimed to detect phytochemicals and metals in medicinal plants collected from hilly areas of Bhimber Azad Jammu and Kashmir, Pakistan. Initially, a physical survey was carried out to select the sampling stations. Fresh flower, leaf and root samples (1-1.5 kg wet weight) of the targeted plants were collected separately in

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polythene bags from pre-decided locations during spring season of the year 2018. The samples were authenticated at the Department of Botany, Mirpur University of Science and Technology (MUST), Mirpur-10250 (AJK), Pakistan. The flowers and leafs were plucked with gloved hands whereas the diamond saw blades purchased from local market were used to cut and collect the root samples. Then, to remove soil particles, the collected plants' parts were washed carefully with tap water and distilled water respectively. The plants' parts were cut into small pieces and left to dry them out in mild sunlight for a number of days. The commercial beater was used to convert dried parts of the plants into coarse powder. The dried and powdered samples packed in pre-washed polythene bags were shifted to the lab for wet digestion and metal analysis.

Figure 1. *Map of district Bhimber, the sampling area*

Digestion of plants' parts for metal analysis

Digestion protocol explained by Sharma et al. (2008) was followed. Various parts of the plant samples (powdered) were digested using the fixed ratio of concentrated H_2SO_4, HNO_3 and $HClO_4$. For this purpose, mixture of conc. sulphuric acid, perchloric acid and nitric acid (1:1:5) was utilised. One g of powdered plant sample taken in beaker was added 15 mL of acid mixture. The sample in beaker was heated for digestion at

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70 °C on hot plate in a fume hood. In order to avoid the evaporation of metal contents, the digestion process was carried out at slow rate. Near to dryness, 10 mL of acid mixture were added again and the temperature was increased slowly upto 90 °C to get around 5-8 mL of clear digested solution. Then, the cooled digested solution in the beaker was added 15 mL of nitric acid (0.05 N) and filtered by using Whatsman filter paper no. 41. The volume of the filtered digest was made upto 30 mL with 0.05 N nitric acid solution and stored at 4 °C in pre-washed, dried polythene bottles for metal analysis (Sharma et al., 2008).

Extraction of the plant samples for analysis of phytochemicals

Two-step extraction procedure was adopted for the investigation of antioxidant activity and phytochemical components. Water and acetone solvents were used in first and second steps for the extraction of hydrophilic and hydrophobic contents respectively.

For water extraction, 1 g powdered plant sample in 10 mL distilled water was centrifuged for 20 min at 6000 rpm and supernatant was shifted to a test tube. The same extraction practice was repeated thrice and each time upper layer was collected in the test tube. The solid residue left after the water extraction was further extracted three times using acetone (1:10 w/v) and the supernatant layer was saved in another test tube. Both the extracts containing phytochemical ingredients were stored at -10 °C to carry out further analysis in the department of Chemistr, Mirpur University of Science and Technology, Mirpur, which is situated at Pakistan country in the cities place category with the GPS coordinates of 73° 45’ 6.3720” E and 33° 8’ 54.2112” N.

Investigation of phenolic contents

The investigation of phenolic contents in water and $(CH_3)_2CO$ concentrates was finished with the assistance of the strategy depicted by Jing et al. (2015). Above all, arranged the ten time’s diluted Folin-ciocalteu by including one mL Folin-ciocalteu, finished the level up to ten mL with distilled H_2O. Around 1 mL of H_2O and $(CH_3)_2CO$ concentrate was mixed with 5 mL of diluted Folin-ciocalteu and 4 mL of 7.5% Na_2CO_3. Before measuring spectrophotometrically the absorbance at 760 nm, left the blend at room temperature to 90 min.

Investigation of flavonoids contents

Recognition of flavonoids was finished with the assistance of strategy altered by Jing et al. (2015). Around 5 mL of H_2O or $(CH_3)_2CO$ concentrates were added into 0.3 mL of 5% $NaNO_2$ for 5 min, and then included 0.3 mL of 10% $AlCl_3$ in the certain blend. Two mL of 1 M NaOH was included the blend to discontinue the response after six min. The surrendered blend was diluted to 10 mL and calculated the absorbance quickly at 510 nm. Rutin was utilized as standard and flavonoids were explained as rutin counterparts.

Investigation of flavonols

Flavonols in the samples were recognized by utilizing the strategy set by Kumaran and Karunakaran (2006). Around 2 mL of H_2O or $(CH_3)_2CO$ concentrate mixed with 2 mL of (50 g/L) CH_3COONa and 2mL of 2% aluminium chloride in the concentrate.

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The blend was permitted to left for 2.5 h and then measured the absorbance at 440 nm. Rutin was utilized as a standard for the detection of flavonols using calibration curve equation y = 0.0007x + 0.3591 (x = concentration of rutin, y = absorbance).

Scavenging activity of DPPH

Scavenging action of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was documented by the strategy portrayed by Yu et al. (2002) and Aoshima et al. (2004). Around 1 mL of the specimen concentrate was added in 2.5 mL of DPPH (0.1 mM in CH_3OH) and held the blend in darkness for 30 min. At 517 nm, vanishing of the shade of DPPH was measured against the clear. The outcomes were measured as *Equation 1:*

$$\% \text{ inhibition} = \frac{\text{A Blank} - \text{A Sample}}{\text{A Blank}} \times 100 \quad \text{(Eq.1)}$$

Scavenging activity of OH˚

The scavenging activity of OH˚ of concentrates of plants in H_2O and $(CH_3)_2CO$ was portrayed by Yu et al. (2004). Around 3 mL of H_2O or $(CH_3)_2CO$ concentrate was mixed in 1 mL of $C_{12}H_8N_2$ (0.04 M), 2 mL phosphate buffer (0.2 M), and 0.04 L of $FeSO_4$ (0.02 M). The reaction was begun by the addition of 0.1 mL of 7.0 mM hydrogen peroxide in the blend, and then measured absorbance at 560 nm. The scavenging activity was evaluated as expressed in *Equation 2:*

$$\text{Scavenging activity} = \frac{\text{A Blank} - \text{A Sample}}{\text{A Blank}} \times 100 \quad \text{(Eq.2)}$$

Scavenging activity of hydrogen peroxide

Aiyegoro and Okoh (2010) reported the method for the measurement of scavenging activity of H_2O_2. The 2.4 mL of hydrogen peroxide solution (4 mM) prepared in 0.1 M phosphate buffer (pH 7.4) was blended with 4 mL extract and incubated for 10 min at room temperature, then measured absorbance at 230 nm against the blank (Reimann and de Caritat, 2005)**.** Scavenging activity was calculated as *Equation 3:*

$$\text{Scavenging activity} = \frac{\text{A Blank} - \text{A Sample}}{\text{A Blank}} \times 100 \quad \text{(Eq.3)}$$

Chelating action of Fe^{+2}

The chelating capacity of Fe^{+2} ions was evaluated via the technique for Dinis et al. (1994). Around 2 mL $FeSO_4$ (0.125 mM) was mixed with 2 mL of water or $CH_3)_2CO$ extracts. Two mL ferrozine (0.3125 mM) was added into the blend to start the reaction and left the blend for ten min. The absorbance was measured at 562 nm against the blank. The accompanying equation was utilized to ascertain chelating action (*Eq. 4*):

$$\text{Chelating Activity} = \frac{(\text{A control} - \text{A Sample})}{(\text{A control})} \times 100 \quad \text{(Eq.4)}$$

Ferric reducing antioxidant power (FRAP)

Hazara et al. described the method for the detection of ferric lessening cancer prevention agent control (Hazara et al., 2008). Around 2 mL of concentrate was blended

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with the two mL phosphate buffer (0.2 M, pH 6.6) and two mL of 0.1% $C_6N_6FeK_3$ and held the mixture for twenty min at 50 °C. Subsequent to twenty min, the reaction was clogged by two mL $C_2HCl_3O_2$ (10%). The supernants were blended with 2 mL of $FeCl_3$ and 2 mL of H_2O and permitted to remain for 20 min and afterward absorbance was measured at 700 nm aligned with the clear. Ascorbic corrosive was utilized as positive control.

Ascorbic acid determination

Ascorbic corrosive substances were controlled by the strategy as expressed by Klein and Perry (1982). Dry water extract was re-extricated for HO_3P (1%, 10 mL) to 45 min and then filtered. 1 mL filtrate was blended in 9 mL $C_{12}H_7NCl_2O_2$ (0.8 g/1000 mL) and then measured absorbance inside thirty min at 515 nm. Ascorbic substances computed from L-ascorbic acid bend (0. 006-0.1 mg/mL; y = 3. 006x + 0. 007; R^2 = 0. 999), and results were communicated as ascorbic corrosive equivalents.

Phosomolybdenium complex assay

Prieto et al. described the method to measure Phosomolybdenium complex of the extracts spectrophotometrically (Prieto et al., 2006). Quickly 2 mL of sample in H_2O or $CH_3)_2CO$ was mixed in 6.6 mL claiming reagent (28 mol/L Na_3PO_4, 0.6 $molL^{-1}$ sulphuric acid and 4 $molL^{-1}$ $(NH_4)_6Mo_7O_{24}$ topped the mixture and incubated at 95 °C for 90 min. After cooling, the absorbance was measured at 695 nm alongside reasonable holding one mL of reagent and also one mL H_2O rather than extracts and after that subjected to the same exploratory conditions. The outcomes from three examinations, each kept running in triplicate, were communicated as the mean of relative antioxidant action (RAA) compared with that of vitamin-C.

Daily intake of metals (DIM)

Information regarding the daily ingestion of therapeutic plants was gathered during the study. DIM was computed as *Equation 5:*

$$DIM = \frac{Cmetal \times CFactor \times Cfood\ intake}{Baverage\ weight} \quad \text{(Eq.5)}$$

C_{metal} stands for metal contents in selected samples, C_{Factor} depicts the conversion factor (value is 0.083), $C_{food\ intake}$ utilization of therapeutic plants which is 100 mg per individual per day and $B_{average\ weight}$ corresponds to the normal body weight within review range which is 50 kg.

Health risk index (HRI)

$$HRI = DIM / RfD \quad \text{(Eq.6)}$$

RfD characterizes the reference oral measurement. The value of health risk index less than one represents the protected mode for nearby populace through intake of plants and the other way around (*Eq. 6*).

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Results and discussion

Flavonoids, flavonols and total phenol contents of the extracts

It has been identified that flavonoids show cell reinforcement endeavour and their outcomes on ethnical nourishment then wellbeing are impressive. Several recent studies have confirmed that phytochemicals including alkaloids, flavonoids, flavonols, phenols, terpenoids and steroids have colossal antioxidant and free radical scavenging activities. Plant extracts rich in polyphenols and essential phytoconstituents have been shown to display persuasive antioxidant and free radical scavenging activities in diverse antioxidant models (Farhan et al., 2012; Amari et al., 2014).

The flavonoids, flavonols and phenols are important as their hydroxyl groups are accountable for antioxidant effects in plants. Highest flavonoid contents were shown by water extract of leaves of *Ajuga bracteosa* (81.56 mg/g) and least contents (12.01 mg/g) were present in roots of *Calotropis procera* (*Table 1*). Leaves and flowers of *Calotropis procera* showed highest flavonol contents in water and acetone extracts respectively (98.98 mg/g, 60.27 mg/g). Least flavonol contents were present in water and acetone extracts of roots of *Ajuga bracteosa* (82.71 mg/g). Phenolic substance are measured as far as gallic equivalents having highest contents in leaves of *Ajuga bracteosa* (66.60 mg/g) followed by leaves of *Calotropis procera* (*Table 1*).

Table 1. *Phytochemical constituents in leaves, flowers and roots of Ajuga bracteosa and Calotropis procera*

Botanical name of therapeutic plants	Part of the plant taken	Local name	Total phenolic contents (mg/g)		Flavonoid contents (mg/g)		Flavonol contents (mg/g)	
			Water	Acetone	Water	Acetone	Water	Acetone
Ajuga bracteosa	Leaves	Hari Boti	66.60	56.10	81.56	40.77	20.48	15.44
Ajuga bracteosa	Flowers	Hari Boti	26.60	24.95	30.21	28.84	47.81	25.74
Ajuga bracteosa	Roots	Hari Boti	60.43	53.63	43.54	31.41	82.71	24.75
Calotropis procera	Flowers	Aak	59.67	21.26	32.62	26.72	60.27	16.83
Calotropis procera	Leaves	Aak	60.6 7	41.20	82.56	14.10	98.98	35.73
Calotropis procera	Roots	Aak	43.17	11.25	12.01	12.54	42.62	23.61

Antioxidant activity

The antioxidants from the leaves, roots and flowers of *Ajuga bracteosa* and *Calotropis procera extracted* in water and acetone are shown in *Table 2*.

The free radical scavenging activity of both hydrophilic and lipophilic antioxidants is estimated by the DPPH radical that is a stable free radical, and has been widely used as a sensitive and rapid tool. DPPH interact with antioxidants that neutralize the free radicals by transferring electrons or hydrogen atoms to DPPH (Archana et al., 2005). Watery extract of flowers of *Ajuga bracteosa* showed highest DPPH scavenging activity (97%) over roots and leaves (96%, 94%). The OH radical has been considered as a highly damaging ROS in free radical pathology, has ability to damage almost every molecule in living cells. The hydroxyl radical scavenging capacity of therapeutic plants is directly related to their antioxidant activity (Uttara et al., 2009). Roots of *Ajuga bracteosa* and leaves of *Calotropis procera* gave the same OH radical and hydrogen peroxide scavenging activity respectively in aqueous extracts (77%), while acetone

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extracts gave the poor OH radical and hydrogen peroxide scavenging activities. The higher antioxidant yield from leaves and blooms of Ajuga bracteosa and Calotropis procera with water solvent prominently indicate viability of this solvent towards antioxidant components from these materials.

Table 2. *Antioxidant activities in leaves, flowers and roots of Ajuga bracteosa and Calotropis procera*

Botanical name of therapeutic plants	Part of the plant taken	Local name	DPPH scavenging activity (%)		OH radical scavenging activity (%)		Hydrogen peroxide radical scavenging activity (%)	
			Water	Acetone	Water	Acetone	Water	Acetone
Ajuga bracteosa	Leaves	Hari Boti	94	81	30	48	23	02
Ajuga bracteosa	Flowers	Hari Boti	57	47	57	15	21	13
Ajuga bracteosa	Roots	Hari Boti	96	88	77	16	40	04
Calotropis procera	Flowers	Aak	94	77	75	14	78	16
Calotropis procera	Leaves	Aak	89	87	59	8	77	51
Calotropis procera	Roots	Aak	73	55	42	06	76	23

Metal chelating action

The chelating of Fe^{+2} by different plant extracts is evaluated via the method utilized by Dinis et al. (1994). Different studies revealed that the reducing power of biologically active compounds relative to their antioxidant action is reflected by the electron donation capability. Antioxidants are reducing agents, and inactivation of oxidants by reductants can be described as a reduction–oxidation (redox) reaction, in which one reaction species is reduced at the expense of the oxidation of the other (Gulcin et al., 2010). In the reducing power assay, the presence of antioxidants in the therapeutic plants would cause the reduction of Fe_3^+ to Fe_2^+ by donating the electron. Aqueous extract of leaves of *Ajuga bracteosa* was the most active extract interfered with the formation of ferrous and ferrozine complex, that is connected with redox metal catalysis incorporates chelating in regards to the metal particles before ferrozine followed by chelating activity of roots and flowers of *Ajuga bracteosa,* then, leaves of *Calotropis procera*. Acetone extracts showed less chelating activity towards Fe^{+2} (*Table 3*).

Table 3. *Fe^{+2} chelating activity and FRAP activity in leaves, flowers and roots of Ajuga bracteosa and Calotropis procera*

Botanical name of therapeutic plants	Part of the plant taken	Local name	Fe^{+2} chelating activity (%)		FRAP activity (%)	
			Water	Acetone	Water	Acetone
Ajuga bracteosa	Leaves	Hari Boti	67	10	68	43
Ajuga bracteosa	Flowers	Hari Boti	33	10	71	50
Ajuga bracteosa	Roots	Hari Boti	56	68	82	62
Calotropis procera	Flowers	Aak	17	21	78	82
Calotropis procera	Leaves	Aak	46	34	69	75
Calotropis procera	Roots	Aak	43	11	32	26

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Table 3 showed the ferric reducing antioxidant power (FRAP) activity in different parts of *Ajuga bracteosa* and *Calotropis procera* in aqueous and acetone extracts. Acetone extracts of *Calotropis procera* showed highest FRAP activity followed by water extracts of *Ajuga bracteosa*.

Phosomolybdenium complex assay and ascorbic acid determination

Phosphomolybdenium assay was expressed as mg AAE/100 g (*Table 4*).

Roots of *Ajuga bracteosa* and flowers of *Calotropis procera* showed significantly higher values in aqueous extract (212.9 mg/g, 207.65 mg/g) respectively. Aqueous extract of flowers of *Ajuga bracteosa* showed the second higher contents (100 mg/g). Lower contents were observed in acetone extracts of flowers of *Ajuga bracteosa* and leaves of *Calotropis procera* (6.0 mg/g, 6.65 mg/g). Significantly differences in ascorbic acid contents among different parts of two plants were recorded (*Table 4*). Flowers of *Ajuga bracteosa* and *Calotropis procera* had the highest and same ascorbic acid contents (0.469 mg/g) followed by roots of *Ajuga bracteosa* (0.379 mg/g). Lower contents were observed in roots of *Calotropis procera* (0.121 mg/g).

Table 4. *Phosomolybdenium complex assay and ascorbic acid contents in leaves, flowers and roots of Ajuga bracteosa and Calotropis procera*

Botanical name of therapeutic plants	Part of the plant taken	Local name	Phosomolybdenium complex assay (mg/g)		Ascorbic acid determination (mg/g)
			Water	Acetone	
Ajuga bracteosa	Leaves	Hari Boti	64.4	38.65	0.261
Ajuga bracteosa	Flowers	Hari Boti	100.15	6.0	0.469
Ajuga bracteosa	Roots	Hari Boti	212.9	13.9	0.379
Calotropis procera	Flowers	Aak	207.65	15.90	0.469
Calotropis procera	Leaves	Aak	38.90	6.65	0.261
Calotropis procera	Roots	Aak	47.20	21.50	0.121

Distribution of metals

Sources of different metals in soil, plants and water are anthropogenic activities and furthermore from parent materials; however it is hard to assess the ordinary foundation groupings of metals in soil, water and plants (Reimann and de Caritat, 2005). Thus it is surveyed that centralization of metals contains both a trademark geochemical partition and anthropogenic exercises (Acosta et al., 2010). The distribution of metals among selected plants is represented in *Table 5*. Except lead, all metals are present in considerable concentrations. Highest mean level is shown by iron followed by zinc. The decreasing order of concentration of metals is as follows; Fe > Zn > Cu > Ni > Cd > Pb. The relationship study was likewise done to evaluate the shared varieties of chose metals in selected plants.

The correlation coefficients of selected metals in medicinal plants are shown in *Table 6*. Compact disc Fe (r = 0.756) and Fe-Cu (r = 0.633) depicted the positive association. Be that as it may, exceptionally solid positive connection was appeared by Pb-Ni (r = 0.839). Strongest connection was appeared by Zn-Fe (r = 0.869) and Zn-Cd (r = 0.851), showing their conceivable basic starting point.

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Table 5. *Concentration (mg/kg) of metal contents in extracts of leaves, flowers and roots of selected medicinal plants (n = 3)*

Samples	Pb	Ni	Zn	Cd	Fe	Cu
Ajuga bracteosa, leaves	BDL	0.092± 0.005	0.449± 0.010	0.104± 0.011	8.678± 0.001	0.084± 0.055
Ajuga bracteosa, flowers	BDL	0.177± 0.035	0.500± 0.013	0.100± 0.051	12.83± 0.025	0.106± 0.012
Ajuga bracteosa, roots	BDL	0.113± 0.031	0.608± 0.004	0.105± 0.005	27.97± 0. 105	0.279± 0.051
Calotropis procera, leaves	0.015± 0.001	0.171± 0. 105	0.277± 0. 501	0.096± 0.012	6.910± 0.001	0.122± 0.001
Calotropis procera flowers	BDL	0.122± 0.010	0.307± 0.050	0.094± 0.016	4.045± 0.018	0.164± 0.005
Calotropis procera roots	BDL	0.136± 0.016	0.421± 0.007	0.097± 0.009	7.281± 0. 105	0.214± 0.004

BDL stands for below detection limit

Table 6. *Correlation co-efficient of selected metals in therapeutic plants*

	Pb	Ni	Zn	Cd	Fe
Ni	**0.839**				
Zn	-0.308	-0.279			
Cd	-0.402	-0.471	**0.851**		
Fe	-0.208	-0.178	**0.869**	**0.756**	
Cu	-0.109	-0.243	0.459	0.160	**0.633**

$P \leq 0.05$ Significant

Cluster investigation of metals was done so as to study the multivariate seizure as appeared as dandrogram in *Figure 2*. Cluster examination gives the huge information on the premise of comparable qualities. Three clusters were observed for selected metals. First cluster exhibited close association of metals (Zn, Fe, and Cd). Cluster of Cu showed mutual relationship with first cluster depicted that the metal concentrations vary because of basic components of the soil minerals and they have lithogenic cause. Third Cluster was appeared by Ni and Pb which also entangled with other clusters revealed that this association may be contributed by the horticultural exercises and additionally dry statement of the suspended particulates.

The quartile circulation of metals in therapeutic plants is shown in *Figure 2*. Symmetric distribution was shown by essential metal i.e. iron. Narrow distribution was shown by Ni, Pb, Cu and Cd. on the ability about dirt towards a multivariable-based cation profession get ready fundamentally liable to physicochemical conditions, for example, pH, temperature and the propinquity of different particles within the dirt structure. Description of health risk assessment is represented in *Table 7*. Health risk index revealed that some metals have safe level in selected plants so that some parts of selected therapeutic plants can be used as drug development and as a supplement to human body. Higher levels of cadmium and iron are investigated by consuming different parts of selected plants.

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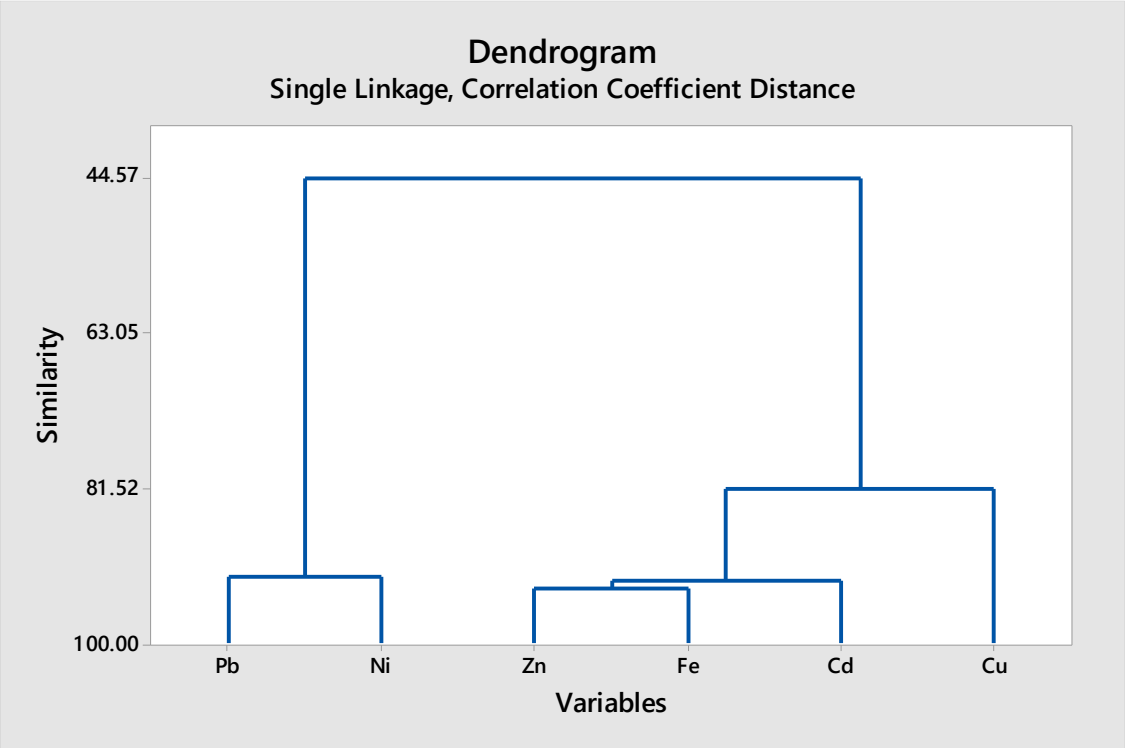

***Figure** 2. Cluster investigation of metals*

***Table 7.** Description of health risk assessment of metals in selected parts of the plants*

RfD	DIM (mg/day)						HRI					
Plants and their parts	*Ajuga bracteosa*			*Calotropis procera*			*Ajuga bracteosa*			*Calotropis procera*		
	L	**R**	**F**	**L**	**R**	**F**	**L**	**R**	**F**	**L**	**R**	**F**
Cu[b] 0.040	0.014	0.046	0.018	0.020	0.055	0.027	0.35	1.15	0.43	0.50	0.88	0.68
Ni[b] 0.020	0.015	0.018	0.029	0.028	0.023	0.202	0.75	0.90	1.47	1.40	1.13	1.01
Cd[b] 0.001	0.012	0.017	0.017	0.016	0.016	0.016	12.4	1.74	**16.6**	0.002	**16**	**15.6**
Zn[b] 0.300	0.075	0.100	0.075	0.046	0.070	0.051	0.25	0.33	0.25	0.153	0.94	0.17
Pb[b] 0.0036	BDL	BDL	BDL	0.002	BDL	BDL	BDL	BDL	BDL	0.555	BDL	BDL
Fe[b] 0.700	1.441	**4.64**	2.13	1.147	1.208	0.671	2.06	**6.63**	3.04	1.63	1.73	0.96

[b]USEPA, 2011, L = leaves, R = roots, F = flowers

Conclusion

This study gives an evaluation that chosen plants can be used as cancer prevention agents. Antioxidant properties and phytochemical contents varied among the water and acetone extracts of selected plants. Among these plants, *Ajuga bracteosa* extricate indicated extremely high antioxidant properties and elevated aggregate phenolic substance. It is demonstrated that flavonoid contents are the significant supporter of the cancer prevention agent properties of these plant extracts. The HRI indicates higher levels of cadmium present in roots and flowers of *Calotropis procera* and also in flowers of *Ajuga bracteosa.* The results also indicate that roots of *Calotropis procera* and flowers of both *Calotropis procera* and *Ajuga bracteosa* also exhibit less

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phytochemical constituents and antioxidant activity. Based upon the study outcomes, it is proposed that the users should avoid taking roots and flowers of *Calotropis procera* and flowers of *Ajuga bracteosa* to cure ailment. Further study is recommended to find and isolate the potential antioxidants to be used in the drug development

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