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Evaluation of *Zanthoxylum armatum* its toxic metal contents and proximate analysis

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Abstract

The objective of this study was to determine the elemental contents and proximate analysis of leaves, bark and fruit of *Zanthoxylum armatum*. The worth of natural medicines cannot be ignored anywhere the globe but the accumulation of toxic heavy metals in access make their uses questionable. In this study the Concentration of various metals like Lead (Pb), Manganese (Mn), Zinc (Zn), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni), Potassium (K) and Sodium (Na) was detected in the leaves, stem bark and fruit of this valuable plant using atomic absorption spectroscopy. The concentration of these quantified metals was compared with established limits and it was proved that this plant is free from toxic level of heavy metals. The presence of Zn, Mn and Cr justify the hypoglycaemic potential of this valuable medicinal plant. Nutritional analysis indicated carbohydrates, proteins, fats and crud fibers in sufficient amount. The current finding encourages the manufacturing of various formulations both as medicinal as well as nutritional supplements from of this important medicinal plant.

Keywords: *Zanthoxylum armatum*, Elemental analysis, Proximate analysis.

Introduction

Most of the countries of the world have been facing malnutrition problems. The absence of protein in human food and animal feed is well predictable. The need of the good quality of proteins has been increasing due to rapid growth of population. It has been listed in literature that in Pakistan the nutrition gap would continue to increase unless well-planned measures are adopted to tackle the situation. It is therefore imperative to increase nutrition production by utilizing all the available ways and means. In addition to increase in conventional production, a lot of work has been done in recent years in discovering new chemical and biological techniques for the production of foods and feeds. High carbohydrate and crude fiber contents suggest the suitability of the plants as animal feed[1]. *Zanthoxylum armatum* is a shrub. Leaflet blades usually with prickles. Leaves are compound, imparipinnate with 3-7 foliolate and pellucid-punctate. Petiole and rachis are winged. It grows wild in foothills starting from about 800m up to 1500m in Malakand, Swat, Dir, Hazara, Buner, Muree hills and Rawalpindi [2]. In Pakistan, it is known as Dambrary, Tamur (Urdu) and Dambara (Pashtu). Its fruits and seeds are edible and used as potherb species. Young shoots are used as toothbrush and useful for curing gum diseases. Fruit is used for curing dyspepsia, Pneumonia, tick infestations and stomachache. Seeds are used as condiment and flavoring agent [3]. Recently we have proved significant results for various pharmacological activities including antipyretic, antimicrobial, cytotoxic, phytotoxic activities using leaves and fruit of this important medicinal plant [3].

As this plant is used traditionally for curative purposes as well as a food item, therefore it was imperative to study its minerals and nutritional composition for both curative and nutritional purposes. In current investigation leaves, bark and fruits of *Z. armatum* were analyzed for elemental and nutritional composition.

Materialand Methods

Plant collection

Fresh plant of *Z. armatum* was collected from Batkhela District Malalkand. Plant materials were cleaned, washed and garbled. Some fresh specimen were pressed, dried, mounted on herbarium sheets, given voucher number Bot. 8815 and kept in the herbarium of Department of Botany, University of Peshawar, Pakistan for ready references.

Reagents and equipment's

Nitric acid (HNO_3), Sulphuric acid (H_2SO_4), Hydrogen per oxide (H_2O_2), Hydrogen fluoride (HF), Perchloric acid ($HClO_4$) and Hydrochloric acid (HCl). All the reagents used were from Merck (Darmstadt, Germany). Pb, Cd, Co and Mn sigma made and Cu, Zn and Fe Aldrich made. Atomic absorption spectrometer (AAS 700 Perkin-Elmer) equipped with MHS-10 (Mercury/Hydride System) flame photometer (Jenway PFP7, UK). Glass wares and plastic equipment were thoroughly washed with water, followed by rinsing with Double distilled water (obtained through Heidolph, Germany water distillation Unit) prior to use under aseptic conditions Using Laminar air flow Cabinet Model 1917 (Forma scientific, USA) in the Department of Botany, University of Peshawar, Pakistan .

Sample preparation

Samples were prepared by wet digestion method [4]. For this purpose 1 g of the respective powder drug was taken in a conical flask, added 10 ml of concentrated HNO_3 (67%) to it and kept overnight (24 h) at room temperature followed by the addition of 4 ml of $HClO_4$ (67%). After 30 minutes, the contents of each flask were heated on hot plate to evaporate, until a clear solution of approximately 1 ml was left. After cooling, the solution was made to a final volume of 100 ml by addition of double distilled water. The solution was then filtered using Whatman # 42 filter paper. The filtrate served as stock solution for each sample. The samples were stored in airtight containers for elemental analysis. Each sample was then analyzed by flame atomic absorption spectrophotometer and flame photometer in triplicate. Calibration standard of each metal was prepared by appropriate dilution of the stock solutions [5]. The sample stock solution was aspired into the flame and the concentration in ppm of each element was calculated by comparing with the standard curve of respective metal.

Nutritional analysis

Plants provide nutritional requirements as they contain protein, carbohydrates, fats and other nutrients, obligatory for growth and development of humans. The proximate analysis of the plant was determined using the published protocols for Ash analysis, Moisture contents, protein, fats and Carbohydrates [6,7].

Statistical analysis

The elemental data was analyzed statistically using Grph pad 5 software. The metal contents were compared with established limits.

Results and Discussion

The Present study deals with elemental and nutritional study of the leaves stem bark and fruit of *Z. armatum*

Effect of elemental analysis

Results of elemental analysis are presented in table 1. In *Z. armatum,* Zn was found 85.3±0.38ppm in leaves, 89.6±0.19 ppm in bark while 87.6±0.19ppm in fruit. Mn concentration detected in the present study was 11.86±0.38, 5.63±0.19 and 2.50± 0.33 ppm in the leaves, bark and fruit respectively, which were in the permissible range for plants (200 ppm). From the results obtained, no significant difference has been observed in concentration of Cr in leaf, bark and fruit of *Z. armatum*. Although fruit (1.66±0.33ppm) has little high values than leaves (1.63±0.19ppm) and bark (1.60±0.33ppm). Copper concentration exceeded the recommended permissible limit (10ppm) in leaf (23.00±0.33 ppm) and in bark and (31.60±0.19 ppm). Fruit of *Z. armatum has* less concentration of Cu (10.00±0.33) as compared to leaf and bark. Iron concentration was detected to be 14.33±0.51ppm in leaf, 15.00±0.33 ppm in bark and 25.67±0.19ppm in fruit of *Z. armatum*, which seems to be in lower concentration than the permissible limit (36-241 ppm). The permissible limit of Ni in plants is 1.5 ppm. The present work revealed that *Z. armatum* bark has maximum amount (8.00±0.33 ppm) of Nickel as compared to its leaves and fruit of Co contents were found higher in leaves (1.70±0.33ppm) than the bark (1.67±0.19 ppm) and fruit (not detected) For this plant. The permissible limit of Pb is 10 ppm (WHO, 1995). In the present study, lead was detected only in the leaves (0.22±0.69 ppm), which is quite lower than the permissible limit while it was not detected in the bark and fruit of *Z. armatum.* In the present study, 916.7±2.19ppm, 570.0±3.33 ppm and 1636.7±2.19 ppm potassium were detected in the leaf, bark and fruit respectively. Adequate concentration of Na in the leaves (132.00±1.33ppm), in the bark (140.67±4.19ppm) and in fruit (141.33±2.19ppm) of *Z. armatum*.

Correlation of elements

From the experimental data it was found that insignificant correlation existed in Zn and Mn (r^2 =0.2500) in the leaves of *Z. armatum* (Fig. 1a). Like leaves, the bark also exhibited very little correlation (r^2 =0.2500) between Zn and Mn (Fig. 2a), while somewhat significan correlation (r^2=0.7500) was detected in fruit for Zn and Mn (Fig. 3a). Similarly significant correlations were existed between Zn and Cr with r^2 values of 0.9463, 0.8292 and 1.000 respectively in leaf, bark and fruit of *Z. armatum*. Correlations between Mn and Cr, in bark showed significant r2value (0.5714) as compared to leaf (r^2 =0.4286) and fruit (r^2 =0.0000) (Fig. 1b, c; Fig. 2b, c; Fig. 3b, c). Similarly Zn/Mn, Zn/Cr and Mn/Cr ratio were also determined in the leaves, bark and fruit of *Z. armatum*. Highest Zn/Mn ratio (35.04) was found in fruit followed by bark (15.91) and the lowest in leaf (7.19). Zn/Cr ratio was found higher in bark as compared to that found in leaves and fruit while highest Mn/Cr ratio existed in leaves (Fig. 4). Correlation and ratios among Zn, Fe and Co were also evaluated. There was a significant correlations between Fe and Co (r^2=0.5714) and Zn and Fe (r^2=0.8929) in the leaves of *Z. armatum* (Fig. 1d, e). Significant correlation were found between Fe and Zn in stem bark with r^2= 0.7500 as compared to Fe and Cr correlation (r^2= 0.7500) (Fig. 2d). No correlation existed among Fe, Zn and Cr in fruit of *Z. armatum* (Fig. 4.39d, e). High Zn/ Fe ratio was recorded in the bark (5.97) as compared to leaf (5.95) and fruit (3.41). Similarly Fe/Co ratio was found higher in bark (8.98) as compared to leaf (8.43) (Fig. 4). Both K^+ and Na^+ ions flowis of extreme importance for life, as body fluid acid-base balance is largely regulated by these ions. Excitation and transmission of nerve impulses in nerve cells during action potential is also under the control of K^+ and Na^+ ions.

Effect of proximate analysis

In the present study too, the nutritional value of *Z. armatum* leaf, bark and fruit was evaluated and the results are presented in (Table 2).Highest carbohydrate value was found in the fruit (59.59±0.06) followed by leaf (45.76±0.08) and bark (38.64±0.11). Protein contents were 16.16±0.05% in leaf, 10.98±0.01% in bark and 7.74±0.04% in fruit. The present analysis for fat contents showed 2.28±0.02%, 1.22±0.05% and 8.90±0.03% fats in the leaf, bark and fruit respectively. In the present study, crude fiber contents were 21.50±0.17% in leaf, 30.83±0.10% in bark and 10.50±0.17% in fruit of *Z. armatum*. The ash values in *Z. armatum* were 11.18±0.09, 13.49±0.01% and 11.90±0.03% in the leaf, bark and fruit respectively. Moisture contents in leaves were 2.90±0.10% in bark 5.03±0.06% in bark and 2.35±0.05% in the fruit.

Zinc (Zn)

Zinc is a non toxic essential micro element, mostly accumulated in human muscles and bones [8]. There are more than 300 Zn dependent proteins and enzymes in human body. Zn plays a vital role in bone strengthening and functioning, cell signaling, hormones release, growth, multiplication of cells [5]. Zn along with Mn and Cr are considered hypoglycemic elements as Zn deficiency cause abnormalities in glucose metabolism. Recommended daily intake of Zn is 15 mg for normal adults while for pregnant and lactating women the daily intake is 20-25 mg. High concentration of Zn in *Z. armatum*

(Table 1) suggests the plant as a rich source of Zn. This plant is reported to play very curative role in combating many health problems, for which one might be to the availability of Zn.

Manganese (Mn)

This element also plays significant role in normal body growth and reproductive function. Its improper supply in diet leads abnormal glucose utilization in the body, leading to Glycemia and Parkinsonism [9]. The presence of high Mn concentration suggested this plant might be a best source of Mn for curing many health problems especially diabetes.

Chromium (Cr)

Chromium is one of the abundant elements on the earth. Chromium acts as an activator for several enzymes necessary for carbohydrates, proteins and cholesterol metabolism. High Cr content may be helpful in heart tonic preparation [10]. Cr is an important constituent of GTF (Glucose Tolerance Factor), which helps in activating the insulin, thus maintaining glucose level in blood. All the three parts of *Z. armatum* crossed the permissible limit (1.5 ppm) of Cr (Table 1), suggesting the plant as a rich source of Cr. This plant might be advised as hypoglycemic agent for treating diabetic patients as well as its possible use in heart tonic preparations as its deficiency causes abnormality in glucose metabolism [11].

Copper (Cu)

Copper is a micronutrient of significant importance as many proteins are copper dependent in human body. Iron deficiency at cellular level is associated with Cu deficiency as it affects Fe transport in the body tissue, thus creating a condition known as hypochromic microcytic anemia [12] The 340–900 µg /day of Cu are the recommended dietary allowance (RDA) for human beings [5]. Copper concentration in the present study exceeded the recommended permissible limit (10 ppm) (Table 1). In the light of above facts, this plant might be helpful in curing hypochromic microcytic anemia and other disorders as it is a very rich source of Cu.

Iron (Fe)

Iron is the most abundant trace element in human body tissues, constituting the core of heamoglobin molecule [12]. Fe is of utmost significance for the metabolic activities of all living organism [5]. The most prevalent nutritional deficiency in humans is reported to be the iron deficiency, especially in females, caused by insufficient dietary intake, excessive menstrual flow or multiple births. Brain functioning is also negatively affected by iron deficiency [13]. Our body takes most of the iron as haemoglobin (57.6%) and non-heme iron complexes (33%) like ferritin and hemosiderin. Fe present in lower concentration (Table 1) than the permissible limit (36-241 ppm). It seems that this plant is not a good source of iron as compared to other minerals.

Nickel (Ni)

Availability of minerals in plants are related to their concentration in the soil, in which they grow.[14] Nickle is easily accumulated in the plants, as it is naturally present in the soil in large amounts. Due to its presence in pancreas and its relation to insulin production, it might play some important role in glucose metabolism. Detective amount of Ni in the present study exceeded (Table 1) the standard permissible limits i.e. 1.5 ppm. This plant might be helpful in formulating pharmaceutical preparations for health hazards like Kidney disorders, cardiovascular problems etc as one of the cause of these diseases is Ni deficiency [15].

Cobalt (Co)

Cobalt is a trace element, essential for plants and present in the tubercles, thus help in nitrogen fixation. The main source of Co is vitamin B12, which can be synthesized by the animals as well as available in their food. The recommended daily intake of Co is 0.13 mg (3 mg of vitamin B12) of which only 50% is absorbed in the intestine [16]. Deficiency of vitamin B12 produces a genetic defect and failure of gastric mucosa and causes disorders related to abnormal functioning of red blood cells. High Cobalt content in synergistic action with iron is suggested for treating blood disorders, especially anemia. In the light of above facts, leaves and bark of this plant might be useful for curing blood related disorders.

Lead (Pb)

Lead (Pb) is a toxic element and its poisoning have serious and even fatal consequences at any age. Children are more susceptible to the ill effects of lead (even in very low oxicity i.e. less than 10 µg/dL), affecting their behavior and devolopment. Lead toxicity includes inhibition of heam biosynthesis, which results in anemia.[5] Quite lower concentration of Pb in the leaves, bark and fruit of *Z. armatum* (Table 1), suggesting its safe usage, with respect to lead.

Sodium (Na)

Sodium is a macronutrient, important for various metabolic process of human body. The most common dietary source is table salt. Na+ with K+ is crucial for life, as their movement play a very crucial role in excitation and transmission of nerve impulse during action potential of nerve cell [17]. Adequate amount of sodium intake is necessary for optimal growth [5]. This plant may be suggested in the nerve disorders caused by sodium deficiency as this plant contains adequate amount of Sodium (Table 1). For plants, no international limit is available, however literature revealed that the daily recommended intake of Na+ is 1–3.8 mg/day.

Table 1: Concentration of various elements in different parts of *Zanthoxylum armatum*

Sample	Zn(ppm)	Mn(ppm)	Cr(ppm)	Cu (ppm)	Fe(ppm)	Ni(ppm)	Co (ppm)	Pb (ppm)	Na (ppm)	K (ppm)
ZL	85.3±0.38	11.86±0.38	1.63±0.19	23.0±0.33	14.33±0.51	7.20±0.33	1.70±0.33	0.22±0.69	132.00±1.33	916.7±2.19
ZB	89.6±0.19	5.63±0.19	1.60±0.33	31.6±0.19	15.00±0.33	8.00±0.33	1.67±0.19	ND	140.67±4.19	570.0±3.33
ZF	87.6±0.19	2.50±0.33	1.66±0.33	10.0±0.33	25.67±0.19	2.37±0.19	ND	ND	141.33±2.19	1636.7±2.19

All values are mean± SEM of three values.

Table 2: Proximate analysis of different parts of *Zanthoxylum armatum*

Sample	Carbohydrates	Proteins	Fats)	Fiber	Ash	Moisture
ZL	45.76±0.08	16.16±0.05	2.28±0.02	21.50±0.17	11.18±0.09	2.90±0.10
ZB	38.64±0.11	10.98±0.01	1.22±0.05	30.83±0.10	13.49±0.01	5.03±0.06
ZF	59.59±0.06	7.74±0.04	8.90±0.03	10.50±0.17	11.90±0.03	2.35±0.05

All values are mean± SEM of three values.

Potassium (K)

Potassium is a macronutrient of both plant and animals and has very crucial status in metabolism. Cellular regulations affected by K^+ concentration include membrane potential regulation, insulin and other hormones secretion, signal transduction and immune response.[18] For plants, no international limit for potassium ion concentration has been reported. However, for human being the average intake of Potassium is 3100 mg/day for adult men and 2300 mg/day for adult women.[19] The results for K signify that this plant is a rich source of potassium.

Correlation ship of trace elements

Correlation exists among the trace elements in plants. Other workers also suggested correlation ship of essential elements like Fe, Zn, Co, Mn and Cr in plants. In the present study, correlations among various elements were carried out [19]. Essential elements like Zinc, manganese and chromium plays an important role in glucose metabolism; therefore they are named as hypoglycemic elements.

Zn like Mn plays important role in wound healing and also act as a strong antioxidant like effect [20]. Similarly it is reported in various research works that Zn, Mn and Cr are Important in maintaining glucose tolerance and secretion of insulin. To ascertain antagonistic and synergistic effects among these trace elements during metabolisms, it is mandatory to find out their correlations and mutual ratios, thus providing bases for their availability in balanced manner.

For diabetics patients, 14.8–28.4 µg/g of Zn may be beneficial and it deficiency was found to be correlated with poor glucose absorption [21]. Similarly iron is reported to facilitate carbohydrate, fat and protein metabolism and control body weight, thus very beneficial for diabetes. Cobalt is also physiologically active in metabolism like Zn and Fe and is required up to 3 µg per day for diabetic patients. Both K^+ and Na^+ ions flowis of extreme importance for life, as body fluid acid-base balance is largely regulated by these ions. The two ions exhibit maximum interdependence for their regulation [22]. During the experimental work, it was found that K^+ and Na^+ correlation was insignificant in the leaves (r^2=0. 0854) while highly significant in bark (r^2=0.7500) and fruit (r^2=0.9146) of *Z. armatum* (Fig. 4.37f, 4.38f and 4.39f respectively). The result shows that K/Na ratio in the fruit (12.47) was highest followed by leaf (6.94) and bark (4.05) (Fig. 4.40). These results indicate that potassium contents were 12.47 times greater in leaves and 8.42 times greater in bark than sodium. These results are well supported by Njoku & Akumefula who reported that K /Na ratio in *Spondias mombin* was 25.5., also obtained more or less the same results for leaf, root and seed of *Cichorium intybus*.

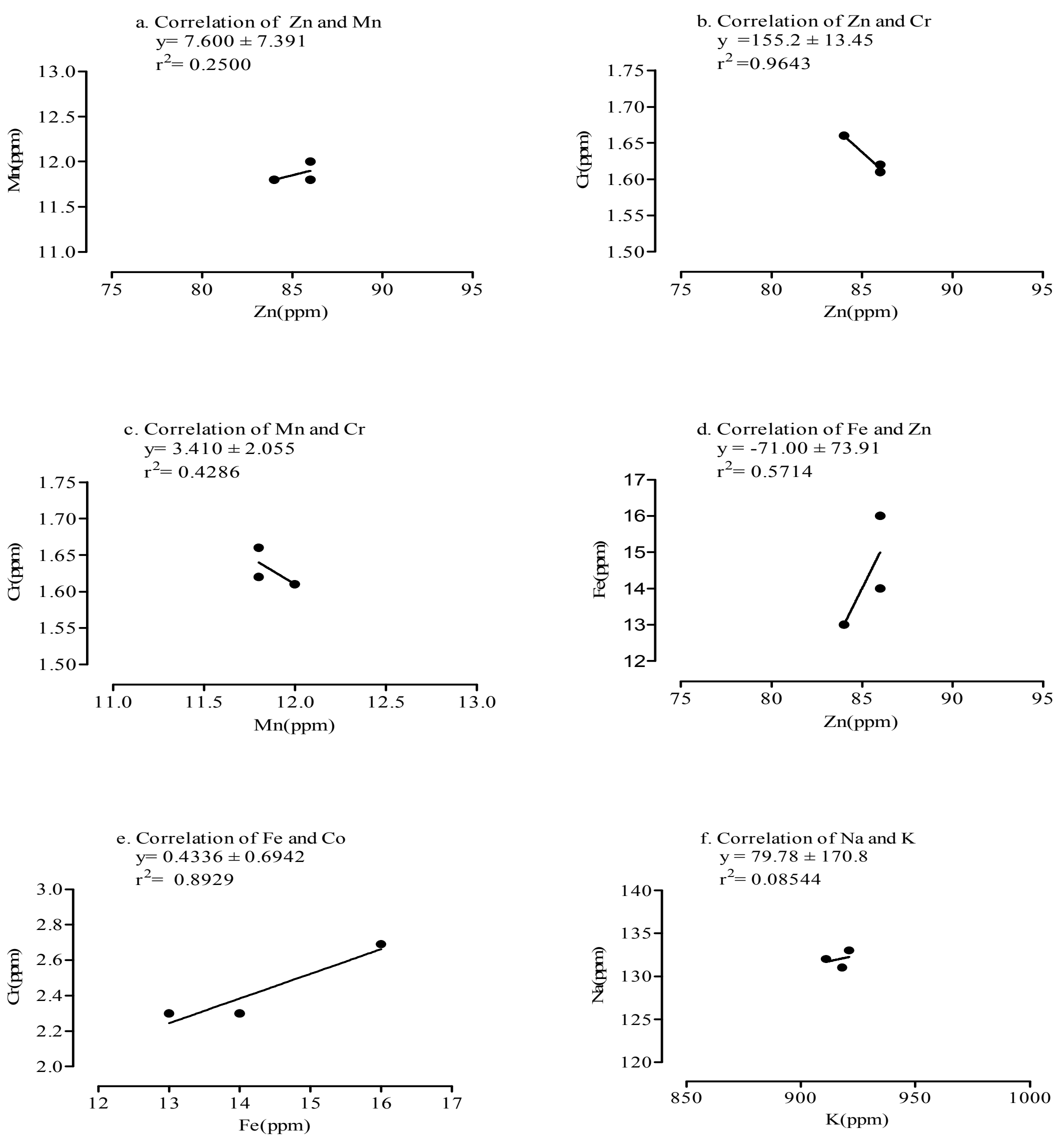

Fig. 1. Correlations of various trace elements in *Zanthoxylum armatum* leaves.

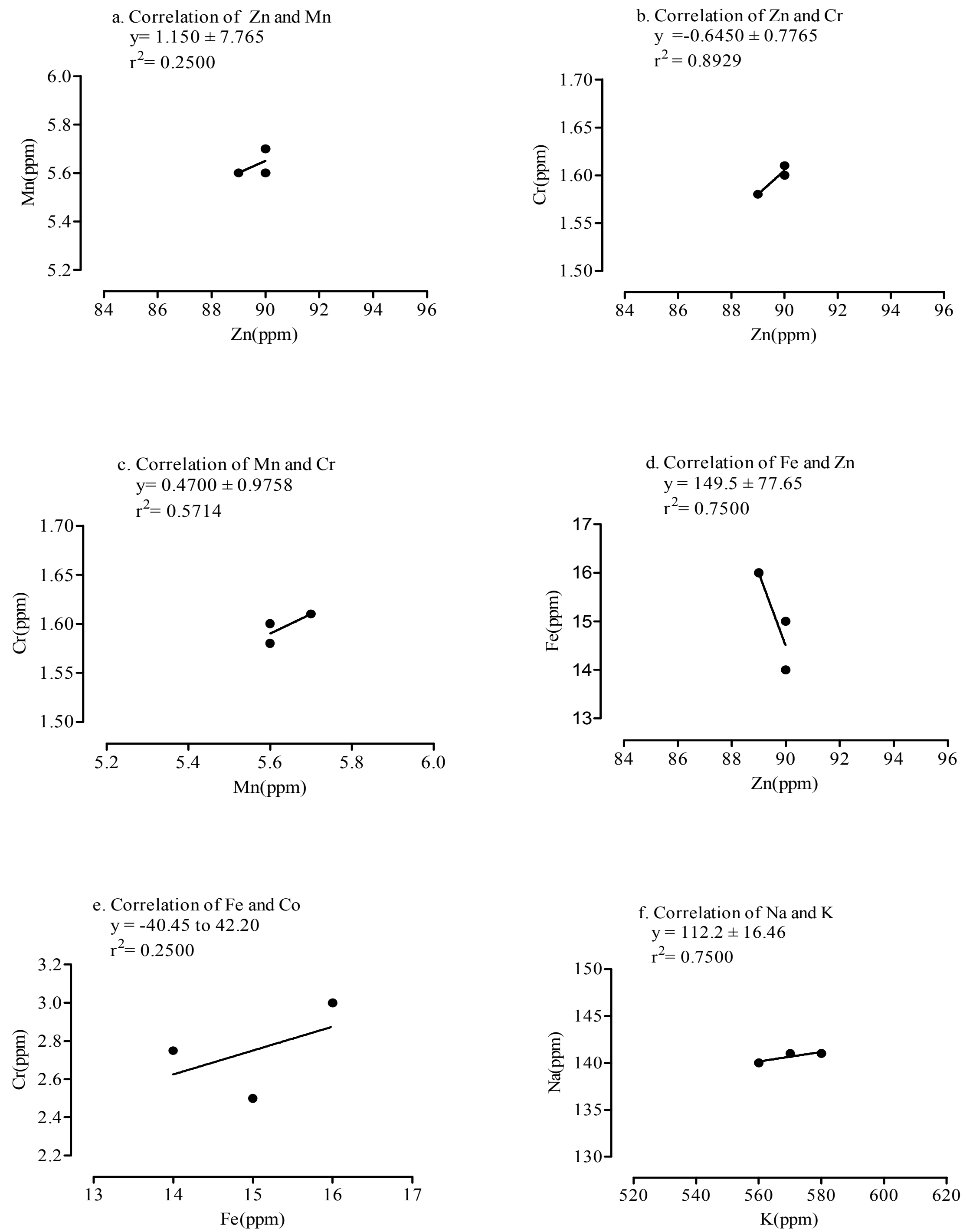

Fig. 2. Correlations of various trace elements in *Zanthoxylum armatum* stem bark.

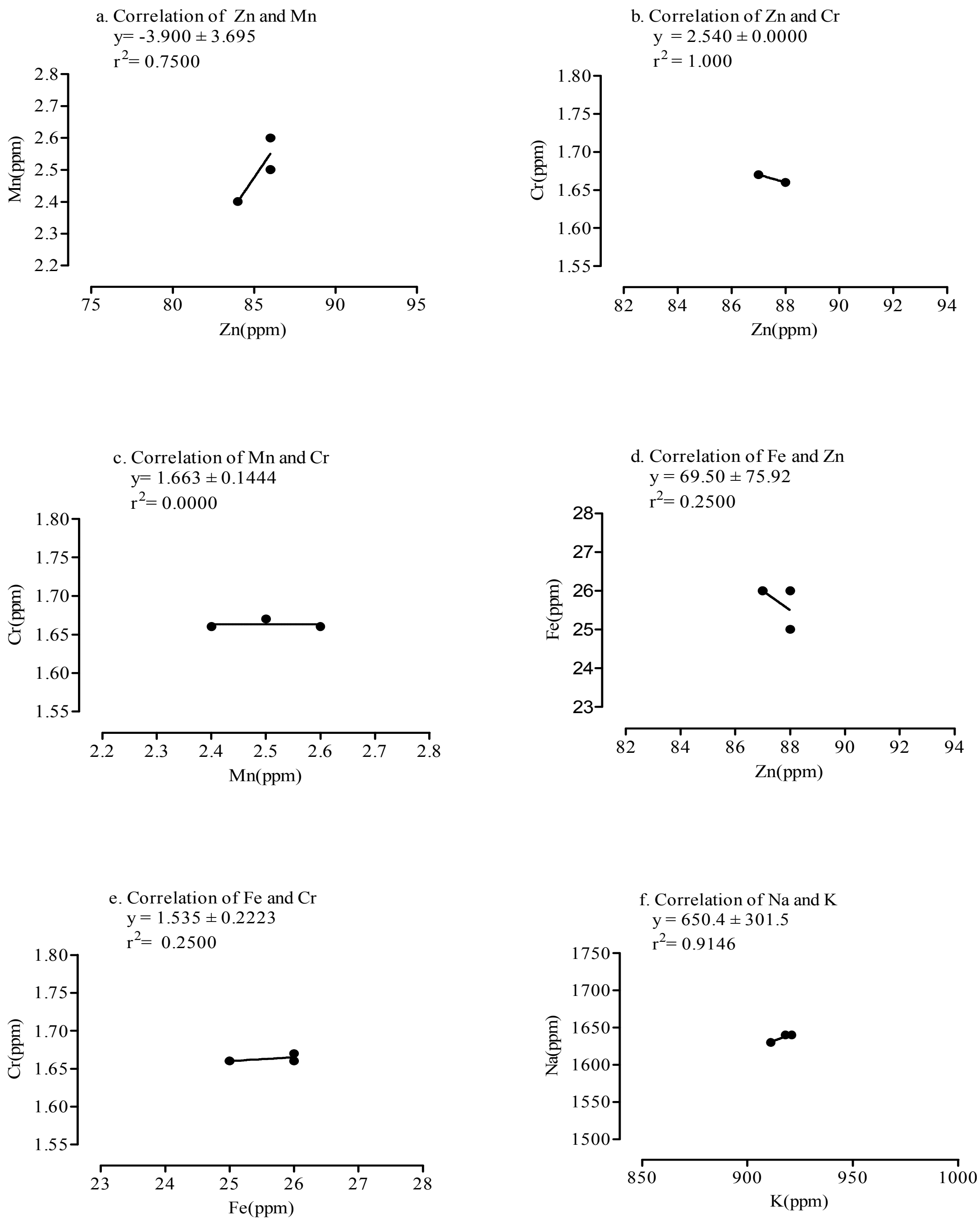

Fig. 3 Correlations of various trace elements in *Zanthoxylum armatum* fruit.

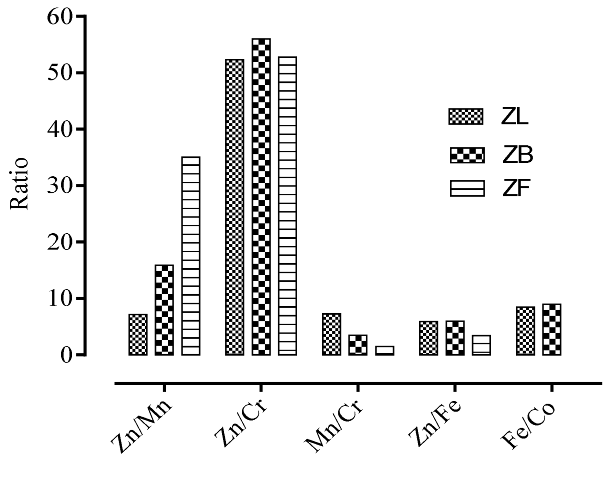

Fig 4: Bars represent ratio among various elements in leaves (ZL), bark (ZB) and fruit (ZF) of Zanthoxylum armatum.

Effect ofproximate analysis

Proximate and nutrient analyses of plants are important for determination of nutritional value. Various medicinal plant species are utilized for curing various ailments. Besides medicinal value, proximate analysis is also important, to ascertain nutritional worth of these plant as well. Carbohydrates are considered the primary source of energy for all organisms, playing nutritional as well as structural role. High carbohydrates contents suggest suitability of the plant as feed. It is imperative to increase protein production by utilizing all the available ways and means as they play both curative and nutritive role. Search for good quality proteins is increasing day by day due to rapid increase in population and health problems. It has been reported that the protein gap in Pakistan would continue to increase unless well-planned measures are adopted to tackle the situation. High protein contents may suggest that this plant might be good source for proteins.

Plant materials containing 1-2% fats, provide energy, which is considered to be significant to human beings, as excess fat consumption results in cardiovascular disorders like atherosclerosis, cancer and aging. The present study about fats suggesting that this plant is safe both for medicinal as well as for nutritional purposes.

Crud fibers are found in higher amount in non starchy materials [23] and are considered good for the treatment of diseases like diabetes, gastrointestinal disorders, obesity and cancer [22]. Our findings suggest that crude fibers amount is well in the reported range and this plant might an important source of dietary crude fibers.

High ash content is an indication of the mineral stuffing available in the plant materials. Ash values ascertained high deposit of minerals in the plant tissue, already explored in the present study.

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