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Nutritional composition, vitamins, minerals and toxic heavy metals analysis of *Digera muricata* (L.) Mart.: A wild edible plant from Peshawar, Pakistan

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This study was aimed to assess the nutritional potential of an unexplored wild edible plant, *Digera muricata* (L.) Mart. commonly used as food in Peshawar, Khyber Pakhtunkhwa, Pakistan. The nutritional, vitamins, minerals and trace elements were investigated, using standard methods of Association of Official Analytical Chemists (AOAC). Nutritional assessment included determination of moisture, ash, lipid, fiber, protein, carbohydrate, and energy. Among the nutrient values, fiber was found to be the highest, followed by ash, carbohydrates, moisture and protein. Lipid was present in very small quantity. The vitamins analyzed were found to have greater value for riboflavin than retinol. Among the macro minerals, potassium was present in high concentration than sodium. The trace elements were assessed using atomic absorption spectrophotometer (AAS) and their decreasing order was Fe>Zn>Mn>Co>Ni. Two toxic metals, Pb and Cd were present in very minute quantities. From the results it was suggested that *D. muricata* (L.) Mart. is a useful plant and can be used as food.

Key words: *Digera muricata* (L.) Mart., nutritional evaluation, vitamins, minerals, Association of Official Analytical Chemists (AOAC).

INTRODUCTION

Nutrition is one of the most important basic needs of humans, for good health, labour productivity, and mental development. Hunger and malnutrition are the problems, increasing in most of the developing countries, due to high rate of population growth, shortage of fertile land and high food prices. Protein deficiency for instance is widespread and has been cited as the most common form of malnutrition in the developing world (Pelletier et al., 1995). Plants have been handled by human societies for food purposes, since time immemorial. It is true that today, human plant food based on mainly twelve crops, which contribute more than 85 to 90% of the total world's caloric intake, but it is also a fact that in many parts of the world, the use of wild plants is not negligible (Prescott-Allen and Prescott-Allen, 1990; Scherrer et al., 2005; Bussmann et al., 2006; Bussmann and Sharon, 2006; Kunwar et al., 2006; Cavender, 2006; Pieroni et al., 2007; Hussain et al., 2009a).

Green leafy vegetables have been recognized for their nutritional importance. They are rich sources of protein contents, ascorbic acid, carotene, folic acid, riboflavin, and minerals like calcium, iron and phosphorus (Kris-Etherton et al., 1988; NRC, 1996; Osler et al., 2001; Sheela et al., 2004). Wild edible green plants are commonly found in countries with rather varied climates. Many researchers have shown several wild species of vegetables fit for human consumption. In some modern cultures people consume wild plants as a normal food

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source, to obtain good amounts of several nutrients and it is widely accepted that leafy green vegetables are significant nutritional sources of minerals (Grau et al., 1989; Kuhnlein, 1990).

Many workers have reported the compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries around the world (Lockeett et al., 2000; Akindahunsi and Salawu, 2005; Edeoga, 2006; Hassan and Umar, 2006; Ekop, 2007; Mohan and Kalidass, 2010; Gafar and Itodo, 2011; Vishwakarma et al., 2011; Valvi and Rathod, 2011; Naryan et al., 2011; Seal, 2011). In Pakistan, wild vegetables are used as food in both urban and rural areas. The researchers have investigated several wild plants of Pakistan, for nutritional composition (Khattak et al., 2006; Imran et al., 2007; Qureshi and Bhatti, 2009; Hussain et al., 2009; Marwat et al., 2010; Jan et al., 2011; Khan et al., 2011). The database of the nutrient and chemical compositions of these plant foods is still incomplete and much work is still needed to be done.

Many of the local wild vegetable materials are under-exploited in Pakistan because of inadequate scientific knowledge of their nutritional potentials. *Digera muricata* is a common weed found during the summer season throughout the plains of Pakistan. It is an annual herb, growing to 20 to 70 cm tall. Stems are simple or branched from the base, nearly hairless. Alternately arranged leaves, 1 to 9 cm long and 0.2 to 5 cm broad, are narrowly linear to broadly ovate. Leaf stalks are long, up to 5 cm, base is narrowed, and the tip pointed. Flowers are hairless, white mixed with pink to carmine or red, usually becoming greenish-white in fruit (Qureshi and Bhatti, 2009; Khan et al., 2011). This plant has been proved very useful for several pharmacological applications in the past. Antioxidant properties of *D*. *muricata* against the CCl_4-induced toxicity have been documented for testis and kidneys (Khan and Ahmed, 2009; Khan et al., 2009). The protective role of *D*. *muricata* against CCl_4-induced oxidative stress in thyroid had been reported by Khan et al. (2011).

In Pakistan and India, the young leaves and shoots are made into curries. To the best of our knowledge, there is no report on nutritional evaluation of *D. muricata* (L.) Mart. This study was designed to evaluate the nutritional status, vitamins, minerals and trace elements analysis of *D. muricata* (L.) Mart. a wild edible plant, commonly consumed as food by the people of Peshawar, Pakistan. Information about the selected plant is given in Table 1 (USDA, 2012).

MATERIALS AND METHODS

Plant

The plant was collected from Peshawar region, agriculture fields of the corresponding author, from July to November, 2011. Fifteen samples were collected from different fields and each sample was studied in triplicate, thus making a total of 45 samples analyzed. Standard methodology was used for collection of plant samples (Humphry, 1993). Specific samples were obtained with the aid of interpreters and field guides. Genus and species was identified by plant taxonomist professor, Iftikhar Alam Khattak, by comparison with herbarium reference materials at Agriculture University Peshawar. The voucher specimen was preserved in Department of Plant Sciences, KUST, Kohat, for future references. The plant leaves were shed dried, pulverized and stored in an airtight container.

Nutritional composition

Moisture was determined by keeping the sample in oven at 100 to 110°C for overnight. The loss in weight was regarded as a measure of moisture content. For ash content, the sample was heated in muffle furnace at 550°C, until white or grayish white ash was obtained. Weight of the ash was noted directly. Crude fibre was measured by treatment of the sample with 1.25% H_2SO_4, 1.25% NaOH and then 1% HNO_3, filtered and washed with hot water after each step. The residue obtained was dried in oven at 130°C and ashed at 550°C in furnace. The loss in weight on ignition was expressed as the content of crude fiber total lipid was extracted from the sample with petroleum ether (60 to 80°C) in a Soxhlet apparatus for about 6 to 8 h. The residual solvent was evaporated in a preweighed beaker and increase in weight of beaker gave total lipid (AOAC, 2000).

Total lipid content was fractionated into saponifiable and non-saponifiable lipids by the saponification of total lipid followed by extraction of non-saponifiable fraction with petroleum ether, 40-60 ºC (AOCS, 1993). Nitrogen content in the sample was estimated by using micro Kjeldahl method and crude protein was calculated by multiplying the evaluated nitrogen by 6.25. The value of total carbohydrate was given by: 100-(percentage of ash + percentage of total lipid + percentage of protein + percentage of crude fibre) (AOAC, 2000). The calorific value was calculated by multiplying the values of total carbohydrate, lipid and protein by the factors 4, 9 and 4, respectively, taking the sum of the products and expressing the result in kilocalories (Guil-Guerrero et al., 1998).

Vitamins analysis

Vitamin A was estimated by extraction with ethanol and then mixing with petroleum ether. The amount in extract was determined by UV-Visible Spectrophotometer (Hitachi U-2000, Japan), at 450 nm, using (Fikselova 2008).

For riboflavin (vitamin B_2), the ethanol extract was added to potassium permanganate and H_2O_2 and allowed to stand over hot water. Then 40% sodium sulphate was added and the absorbance was measured at 510 nm by spectrophotometer (James, 1995).

Minerals and toxic heavy metals analysis

Two macro minerals sodium and potassium were estimated by using flame photometer (Model 410 Corning, Germany). Standard solution of each was used for calibration of the instrument before analysis (AOAC, 2000). The micro minerals including Zn, Cu, Ni, Mn, and Fe along with toxic heavy metals Pb and Cd, were determined, by wet digestion of the sample followed by analysis using atomic absorption spectrophotometer (A-Analyst 700 Perkin Elmer/USA) equipped with standard burner, air acetylene flame and hollow cathode lamps, as radiation source (Indrayan et al., 2005).

Statistical analysis

The data obtained from three replicates were analyzed by ANOVA

Table 1. Description of the sample plant analyzed.

Vernacular names	Scientific classification	
English, False amaranth; Urdu, Lesua; Pashtu, Sur gulae; Hindi, Latmahuria; Sanskrit, Aranya; Marathi, Gitana; Telugu, Chenchhali koora; Arabic, Alqattoeqt - alkazebat	Kingdom	*Plantae*
	Subkingdom	Tracheobionta
	Super division	Spermatophyta
	Division	*Magnoliophyta*
	Class	*Magnoliopsida*
	Subclass	*Caryophyllidae*
	Order	*Caryophyllales*
	Family	*Amaranthaceae*
	Genus	*Digera*
	Species	*Digera muricata (L.) Mart.*

Table 2. Nutritional composition of *Digera muricata* (L.) Mart.

S/N	Nutritional parameter		Quantity		
			Mg/g±SD	%	mg/kg
1	Moisture		$139^{b}±3.2$	13.9±0.3	$1.4×10^{5}$
2	Ash		$181^{b}±4.2$	18.1±0.4	$1.8×10^{5}$
3	Lipid	Total lipid	$50^{a}±2.2$	5.0±0.2	$5×10^{4}$
4		Saponifiable	$32^{a}±1.2$	3.2±0.1	$3.2×10^{4}$
5		Non saponifiable	$18^{a}±0.8$	1.8±0.1	$1.8×10^{4}$
6	Total Protein		$88^{a}±2.6$	8.8±0.3	$8.8×10^{4}$
7	Fiber		$410^{b}±3.8$	41±0.4	$4.\ 1×10^{5}$
8	Carbohydrate		$133^{b}±2.7$	13.3±0.3	$1.3×10^{5}$
9	Energy (kcal/100 g)			$1.4 × 10^{2}$	

$^{a}P<0.05$; $^{b}P>0.05$.

using the SPSS statistical package program, and the differences between the means were compared using the Duncan's multiple range tests at the significance level of 0.05. Values for the same parameter among various samples were represented by superscript a or b, when $p<0.05$ or $p>0.05$ respectively (Tables 2, 3 and 4).

RESULTS AND DISCUSSION

Nutritional composition

Moisture in food is a source of water and is considered necessary that 20% of the total water consumption must come from food moisture (FNB, 2005). The average moisture content of the sample leaves studied was 13.88% (Table 2), very low as compared to other wild edible plants such as *Amaranthus viridus*, *Chenopodium murale*, *Nasturtium officinale* and *Scandix pecten-veneris* with 88.90, 89.50, 90.54 and 81.31%, respectively (Imran et al., 2007). However, it was higher than some common leafy vegetables such as *Xanthosem sagittifolum* (13.17%) and *Adansonia digitata* (9.5%) (Ladan et al., 1996). Ash content is the index of mineral contents in plants such as calcium, sodium, potassium, nickel and zinc. The ash content of the sample studied was 18.14% (Table 2), which showed that the leaves were rich in minerals. The value obtained was higher as compared to 1.8% reported in sweet potato leaves, and 5% in *Tribulus terrestris* leaves, 1.85% in *A. viridus* leaves, 2.70% in *C. murale* leaves, 1.77 and 3.10%, in *N. officinale* and *S. pecten-veneris* leaves respectively. But lower than 19.61% in *Amaranthus hybridus* leaves (Imran et al., 2007).

The crude protein content of the sample was found to be 8.75% (Table 2). This value was higher as compared to 2.11% in *A. viridus,* 2.98% in *C. murale* leaves, 2.76% in *N. officinale*, 6.30% in water spinach and 6.40% in *Momordica foecide* leaves (Imran et al., 2007). But lower than 11.29% in balsam apple leaves, 24.85% in sweet potato leaves, *Piper guineense* and *Talinum triangulare* with values of 29.78 and 31.00%, respectively (Akindahunsi and Salawu, 2005). Among the reported wild edible plants of the same family, sample leaves contain higher crude protein value. According to the WHO recommended dietary allowance (RDA) of protein, for children, adult male and adult female is 28, 63 and 50 g, respectively (Akindahunsi and Salawu, 2005), while in Pakistan the average protein intake is 43.4 g/day (Figure 1) (NNSP, 2011). As the plant protein is also considered

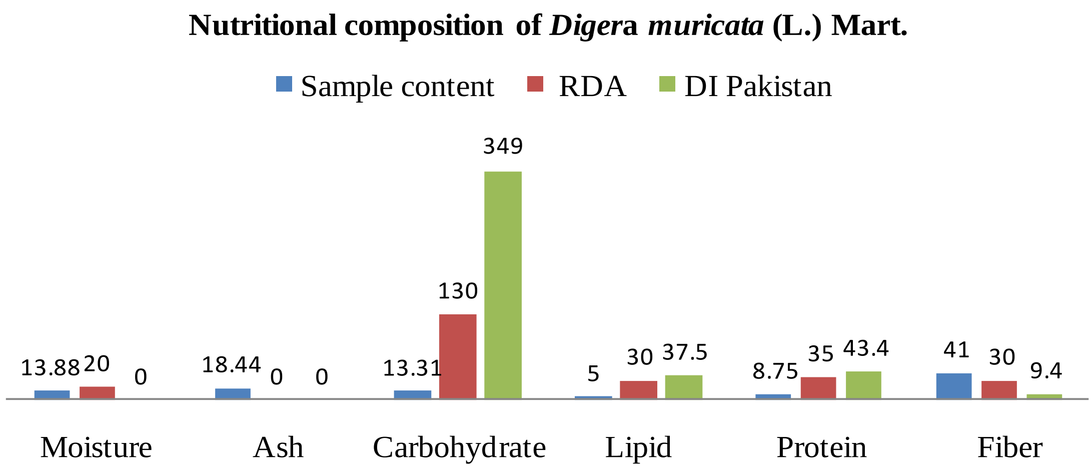

Figure 1. Nutritional composition in comparison with RDA and average nutrient intake in Pakistan.

Table 3. Vitamins constituents of *Digera muricata* (L.) Mart.

S/N	Vitamin	Quantity		
		mg/g±SD	%	mg/kg
1	Vitamin A	0.0115 [a]±0.013	1.15×10^{-3}	11.5
2	Riboflavin (Vit B_2)	0.0204[a]±0.015	2.04×10^{-3}	20.4

[a]P<0.05

Table 4. Minerals and toxic heavy metals of *Digera muricata* (L.) Mart.

S/N	Mineral	Quantity		
		mg/g	%	mg/kg±SD
	Macro-minerals			
1	Sodium	0.08	7.60×10^{-3}	76.00[b]±4.0
2	Potassium	0.76	7.60×10^{-2}	760.0[b]±23
	Micro-minerals			
3	Zinc	2.98×10^{-3}	2.30×10^{-4}	2.298[b]±0.07
4	Copper	6.29×10^{-4}	6.29×10^{-5}	0.629[a]±0.02
5	Iron	2.08×10^{-2}	2.08×10^{-3}	20.75[a]±0.61
6	Manganese	1.71×10^{-3}	1.71×10^{-4}	1.706[b] ±0.06
7	Nickel	2.30×10^{-4}	2.30×10^{-5}	0.230 [a]±0.002
	Toxic heavy metals			
8	Lead	5.06×10^{-4}	5.06×10^{-5}	0.506[a] ±0.01
9	Cadmium	1.90×10^{-5}	1.90×10^{-6}	0.019[a]±0.002

[a]P<0.05; [b]P>0.05.

as low biological value also, so for 100 g of *D. muricata* (L.) Mart. leaves to provide 8.75 g of proteins indicate that the sample leaves are a good source of daily proteins.

Lipid in food is considered as a chief source of storage form of energy, essential fatty acids and fat soluble vitamins and precursors of vitamins. The sample leaves contained 5.0 % crude lipid (Table 1). It was lower than 11 % in water spinach leaves, 12 % in *Senna obtusfolia*, 11 % in *Amaranthus caudatus* leaves, 28.2 % in *Centilla asiatica* leaves, 29 % in *Bahunian purpurea* leaves, and 60% in *Amaranthus hybridus*, but higher than 0.47% in *Amaranthus viridus*, 0.54% in *Chenopodium murale* and 0.63 % in *Scandex pectenveneris* leaves (Imran et al., 2007). The crude fiber content of sample leaves was 41 % which was higher, compared to 7.20 % in sweet potato leaves, 13 % in *Tribubus terristris* leaves, 29.0 % in balsam apple leaves, 1.93 % in *Amaranthus viridus,* 3.82 % in *Scandex pectenveneris* leaves (Akindahunsi and Salawu 2005). Dietary fiber helps to reduce serum cholesterol level, risk of coronary heart diseases, colon and breast cancer and hypertension. The high level of fiber in diet can cause intestinal irritation, lower digestibility, difficult absorption of minerals found in plant and overall decrease nutrient utilization (Imran et al., 2007).

The carbohydrate content of sample leaves was 13.31%, considerably low when compared with other wild edible plants such as *A. caudatus* leaves (61.03%), 55.67% in *T. terrestris,* 54.2% in water spinach leaves, 75% in sweet potato leaves, 82.8% in *Corchorus tridens* leaves. But higher when compared with *A. viridus*, *C.*

murale, *N. officinale* and *S. pecten-veneris* leaves, that is, 4.74, 3.41, 3.38 and 7.32%, respectively (Imran et al., 2007). Carbohydrates are principal and indispensible source of energy. The RDA for carbohydrates is 130 g (FAO, 1998), while in Pakistan 349 g of carbohydrate intake is reported (Figure 1) (Ministry of Health and Nutrition, 1994). Due to carbohydrates content sample plant can be a good food source.

The 100 g of *D. muricata* (L.) Mart. provide 140 kcal of energy. This reveals that the sample plant can contribute meaningfully to the daily energy requirement of a person. The caloric value of *D. muricata* (L.) Mart. is high as compared to *A. viridus* (31.63 kCal), *S. pecten-veneris* (50.23 kcal) (Imran et al., 2007), 134.6 Kcal of *Aegle marmelos.* But lower when compared with *A. caudatus* 326.7 Kcal, *Dioscorea bulbifera* 304.7 kcal and 333.1 kcal of *Ficus bengalensis* (Imran et al., 2007).

Vitamin analysis

The sample contained 1.15 mg / 100 g of vitamin A (Table 3). The RDA for vitamin A is 1.5 mg / 100 g (Figure 2). The result showed that the sample was a good source of vitamin A. Vitamin A is necessary for vision process and also plays a role in skin mucosa, normal reproductive capabilities and is an important anti oxidant (FAO 2001). The sample contained 2.04 mg /100 g of vitamin $B_{2.}$ The RDA for riboflavin is 1.7 mg. The results showed that it was a rich source of riboflavin. Riboflavin is present in body as co – enzyme which acts as hydrogen acceptor in amino acid metabolism (FAO 2001). The riboflavin content was lower than *sonchus eruca* and *sonchus asper* (Hussain et al., 2010).

Minerals analysis

Sodium maintains fluid volume outside the cell thus normalize the cell functions. The sodium content of the sample was 76 mg/kg (Table 4), slightly low as compared to that reported for *T. terrestris* leaves (50 mg/g) and 450 mg/kg in *S. obtusifolia* but quite high in comparison to *Asparagus officinalis* (0.184 mg/kg) and *Momordica dioica* (0.151 mg/kg) (Khan et al., 2011). The RDA for sodium is 500 mg for adults (Figure 3). The plant sample leaves can be good source of food for hypertensive patients (FAO, 2001). Potassium content of the sample was 760 mg/kg (Table 4), high as compared to other green leafy vegetables as 64.2 mg/kg was found in *Diospyros mespiliformis,* 1.09 mg/kg in *A. officinalis,* 0.825 mg/kg in *M. dioica* and 4.409 mg/kg in *Indigofera astragalina* (Khan et al., 2011). The RDA for potassium is 2500 mg for adults (Figure 3), and the sample contributes 30% to RDA, meaning the good source that can contribute to the diet of hypertensive patients (FAO, 2001).

Microminerals analysis

The copper content of plant sample was 0.629 mg/kg (Table 3), which is higher as compared to 0.1 mg/kg in *D. mespiliformis* and 0.25 mg/kg in *Ficus bengalensis,* but lower when compared with 12.8 mg/kg in *T. terrestris* leaves and 5.0 mg/kg in *Cassia siamea* leaves (Khan et al., 2011; Gafar and Itodo, 2011). The RDA value for copper is 1 to 3 mg for adult (Figure 4). Copper contributes a role in hemoglobin formation and plays role in iron and energy metabolism (FAO, 2001). The zinc content of the sample leaves was found to be 2.2984 mg/kg, higher as compared to 0.200 mg/kg in *D. mespiliformis,* 1 mg/kg in *T. terristris* leaves but lower when compared with 68.5 mg/kg in *C. siamea* (Khan et al., 2011; Gafar and Itodo, 2011). Zinc plays a vital role in gene expression, regulation of cellular growth and participates as a cofactor of many enzymes. It also plays an important role in motility of sperm during liquation and mating. The RDA of zinc is 12 to 15 mg for adults (FAO, 2001).

Iron content of the sample was 20.75 mg/kg, higher than other vegetables, but lower than *Cassia siamea* 700 mg/kg (Khan et al., 2011). The RDA value of iron is 10 to 15 mg/100 g. This sample is a good source of iron. Iron is required for hemoglobin formation and its deficiency leads to anemia (FAO, 2001). Manganese content of plant sample was 1.706 mg/kg which is lower than 9.8 to 38 mg/kg reported in some leafy vegetables and 116 mg/kg in balsam apple leaves (Khan et al., 2011). The RDA for manganese is 2 to 5 mg. The result showed that *D. muricata* L. is a good source to provide daily manganese. Manganese is a co-factor for many enzymes which take part in glucose and amino acid metabolism (FAO, 2001). The amount of nickel present was 0.203 mg/kg in the sample. This quantity was quite lower when compared with other edible wild plants like *F. bengalensis* 1.14 mg/kg (Gafar and Itodo, 2011). Nickel is needed in very small amount to the body. The health benefits of Ni are healthy skin and optimal growth and also take part in iron metabolism. Higher quantity leads to toxicity (Gafar and Itodo, 2011).

Toxic heavy metals analysis

The lead content of sample was 0.506 mg/kg (Table 4), higher as compared to *F. bengalensis* (0.25 mg/kg). Lead is toxic and non essential element for human body as it causes rise of blood pressure, kidney damage, miscarriage, subtle abortion, brain damage, decline fertility of men through sperm damage and diminishes learning abilities due to neuron damaging actions (Gafar and Itodo, 2011). Cadmium concentration of *D. muricata* (L.) Mart. was 0.019 mg/kg higher as compared to *F. bengalensis* 0.017 mg/kg and lower than other usual edible plants. Cadmium is highly toxic for a body and it

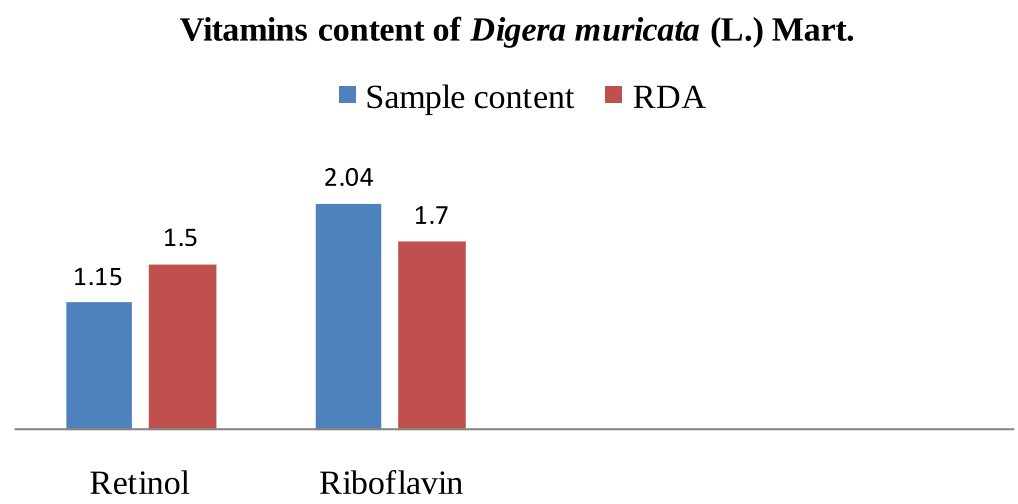

Figure 2. Vitamins content in comparison with RDA.

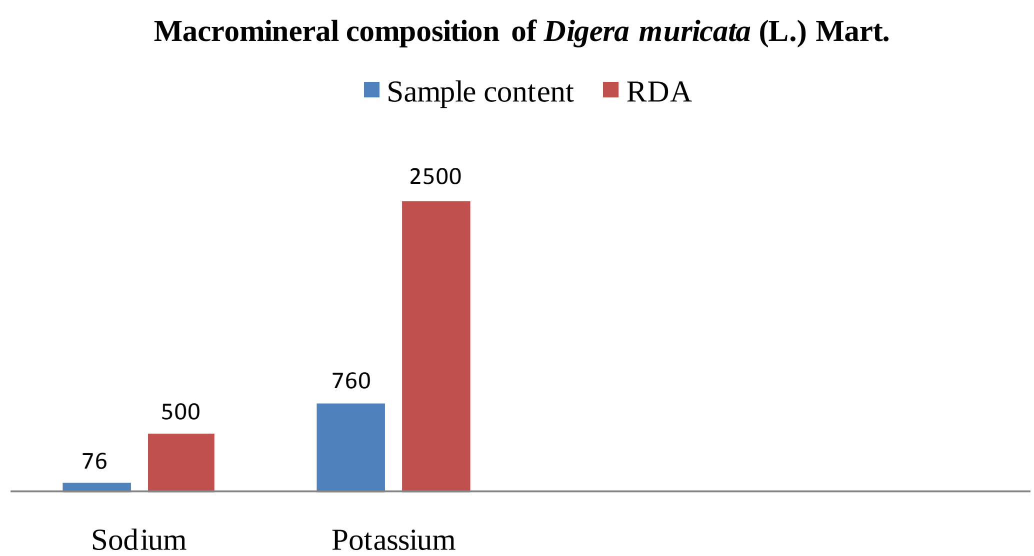

Figure 3. Sodium and potassium contents in comparison with RDA.

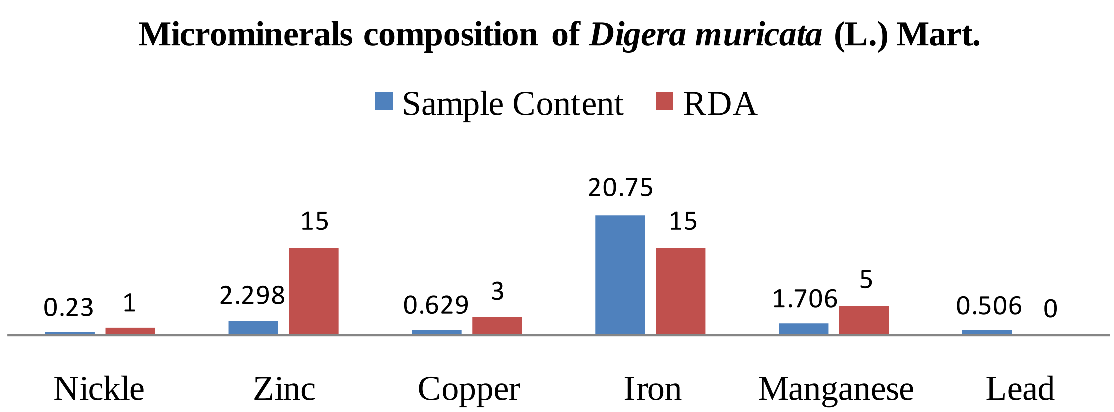

Figure 4. Microminerals in comparison with RDA.

cause a several health hazards, including cell death and cell proliferation (Gafar and Itodo, 2011).

Conclusions

D. muricata (L.) Mart. has good proximate values of moisture, fiber, proteins, and carbohydrates. The macro minerals (Na and K), micro minerals (Fe, Cu, Ni and Mn), and vitamins (A and B_2) are also present in appreciable quantity. The toxic metals like Pb and Cd are present in very minute amount and therefore do not pose any threat to health. These results are making the plant a good source of food and can be recommended for edible uses.

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