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Determination of Selected Metals in Leaf and Root Bark of Malva Parviflora

Mesfin Medihin Ododo* Bezabih Kelta Wabalo
Department of Chemistry, Wolaita Sodo University, Wolaita Sodo, Ethiopia

Abstract
In Ethiopia, *Malva parviflora* (family: Malvaceae) leaf is used as a vegetable and its root bark is used to treat furuncles, carbuncles, wound infections and other related ailments. However, no research has been done to analyze essential and toxic metals in leaf part and the metals having antibacterial and wound healing activities in root bark of this plant. Thus, the present study was aimed to determine concentration of K, Na, Mg, Ca, Fe, Zn, Cu, Cd, Pb, and Ni in leaf par; and Mg, Ca, Fe, Zn, Cu, Cd, Pb, Ni, and Co in root bark of *Malva parviflora*. The results revealed that the leaf part contains high amounts of Na, K, Mg, and Ca. Similarly, high concentrations of Mg and Ca were detected in the root bark. Appreciable amounts of Fe and Zn, and low amount of Cu were detected in both leaf part and root bark. Concentrations of Co, Ni, Cd, and Pb were below the method detection limit. Thus, leaf of *Malva parviflora* is a good source of essential nutrients (Na, K, Mg, Ca, Fe, and Zn) and the presence of Mg, Ca, Fe, Zn and Cu might contribute to the therapeutic action of root bark of *Malva parviflora*.
Keywords: *Malva parviflora*, Vegetable, Medicinal plants, Metals
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1. Background

Malva parviflora (family: Malvaceae) is also commonly known as *Uka* (in Wolaita language) in Ethiopia. It is native to Asia, Northern Africa, and Europe and widely naturalized elsewhere. The plant often grows up to 40 inch in agricultural farmlands and waste places. *Malva parviflora* is an annual, biennial or perennial herb plant. It has a deep strong tap root system, dark green leaves, flowers that emerge from the base of the leaf stalks, and fruits that are sectioned into lobes (Ododo *et al.*, 2016).

Food crops especially vegetables demands have been highly increased due to rapid growth of human population, and wild vegetables are used as alternatives crops to meet the food demands (Hussain *et al.*, 2010). *Malva parviflora* **leaf** is cooked like spinach in Mexico (Sharma and Ali, 1999) and raw or cooked as a potherb in Ethiopia. A mild pleasant flavour, it makes a very acceptable alternative to lettuce in salads or cabbage. The beneficial health and nutrition values of leaf of *Malva parviflora*, for human consumption have been claimed by local users in rural area of Ethiopia. Raw or cooked immature seeds are also used as a vegetable.

Many medicinal plants are being used for both medicinal and nutritional purpose in different countries. They are considered as good source for providing essential elements, and thus the elements contribute therapeutic action of medicinal plants (Selvaraju *et al.*, 2011). In the rural areas of Ethiopia, the root bark of *Malva parviflora* is used in herbal medicine very effective to heal furuncles, carbuncles and wound infections. The minor skin infections such as furuncles, carbuncles are usually caused by *Staphylococcus aureus* bacterium (Chakraborty *et al.*, 2012), and *Escherichia coli* strains cause wound infections (Mos *et al.*, 2010).

The scientific investigation of metals in the leaf part is important to know the nutritional values and their toxicity level. The metals contribution on therapeutic action of medicinal plants also should have been evaluated. Thus, study was aimed to determine concentration of selected metals in both leaf and root bark parts of *Malva parviflora*.

2. Materials and Methods

2.1 Description of the Study area

The study was carried out in around Shanto Town, Damot Pulassa *Woreda*, Wolaita Zone, Ethiopia. Damot Pulasa *Woreda* is one of the 12 districts in Wolaita Zone. Shanto is an administrative town of Damot Pulasa *Woreda* and located about 330 km from Addis Ababa, the capital city of Ethiopia to the southwest. It is found between latitude $07^0$01'28.2"N and longitude $037^0$55'09.9"E and lie at an altitudinal range of 1820-1880 m above sea level. The annual mean minimum and maximum temperatures are about 15.5 and 24.5^{o}C respectively. The total mean annual rainfall is 1000 to 1270 mm. The soil type of the study area is grouped under black basaltic soil.

2.2 Apparatus and Glasswares

A drying oven (Digital Heat, JP Selecta, Spain), mortar, pestle, precision balance (PGW 253i (ADAM), Switzerland), Kjeldahl digestion apparatus (BUCHI, Switzerland), and Whatman No. 42 filter paper with a 0.45-µm were used.

2.3 Reagents and Instruments

Nitric acid (HNO_3, 69–72%) (RANKEM, India), Analytical grade metal salts of Na, K, Ca, Mg, Fe, Co, Ni, Cu, Zn, Cd and Pb and deionized water. Flame photometer (model CL 378, India) and flame atomic absorption spectrophotometer (model, PG-990 AAS, UK) were used to determine concentration of metals.

2.4 Pretreatment of Glasswares

To avoid contamination, all glasswares required for experiments were first washed with cold tap water using a detergent and then soaked in 20% (v/v) HNO_3 for 48 hours, and finally rinsed with deionized water.

2.5 Collection of the Samples

Fresh plant materials were randomly collected from agricultural farmlands in the month of October, 2012 towards the end of the rainy season. Both leaf and root samples collected were kept separately in polyethylene plastic bags.

2.6 Sample Pretreatment, Drying and Grinding

The root materials were washed with tap water followed by deionized water to eliminate attached soil particles. The bark was the separated from the root and chopped into small pieces using a stainless steel knife. After drying in air, the sample was dried in an oven at 60 ºC for 12 hr to assure complete removal of moisture. It was then grinded using mortar and pestle, sieved (2 mm) to obtain uniform particle size. Both leaf and root bark powders were weighed and then stored in clean dried plastic bottles at room temperature for further analysis.

2.7 Wet Digestion of Samples

The wet sample digestion was carried out using Kjeldahl digestion apparatus with a reflux condenser for both leaf and root bark samples. Three parameters: temperature (275°C), time (3 hrs) and reagent volume (15 mL HNO_3) were optimized. Applying the optimized conditions, 1 g of each sample was digested for analysis. The digestion was carried out in triplicate for each sample. The digested solutions were allowed to cool at room temperature for 30 min. To the cooled clear yellow solutions, 20 mL of deionized water was added, and solutions were then shaken to dissolve precipitates remaining on the wall of the digestion apparatus. Then, solutions were filtered into 100 mL volumetric flasks through a Whatman No. 42 filter paper with a 0.45-µm to remove the impurities for metals analysis. The solutions were finally diluted to the mark with deionized water and kept in room temperature until analysis.

2.8 Constructing Calibration curves

To construct calibration curves, diluted standard solutions were prepared from their respective stock standard solutions (1000 ppm) which were already-made by dissolving appropriate amounts of the their respective metal salts of analytical grade (purity 99.9%) in HNO_3 and diluting with deionized water. Preparation of the diluted standard solutions for each metal was depending upon the linear working range (Table 1). The absorbance of each solution was measured and calibration curves were constructed separately for selected metals to determine correlation coefficient (r^2).

Table 1: Standard solutions used for preparing calibration curves

Concentration (ppm) of calibration standard solutions for each metal											
Metal	**Na**	**K**	**Mg**	**Ca**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
Concentration level (ppm)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.25	0.25	0.25	0.50
	10.00	10.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.50	1.00
	15.00	15.00	2.00	2.00	2.00	2.00	2.00	1.00	1.00	1.00	2.00
	20.00	20.00	3.00	3.00	3.00	3.00	3.00	1.50	1.50	1.50	4.00
	25.00	25.00	4.00	4.00	-	4.00	-	2.00	-	2.00	8.00

2.9 Recovery Test

The optimized procedure was validated by spiking each of triplicate leaf and root bark samples with the known concentration of standard metal solutions using a pipette (Table 2). The spiked samples were then digested using optimized procedure and analyzed to calculate percent recoveries.

Table 2: The spiked concentrations levels of selected metals

Metal	**Na**	**K**	**Mg**	**Ca**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
Concentration (ppm) spiked	6	12	3	3	1	1	1	1	1	1	1

2.10 Method Detection Limit

Method detection limits (3SD, where SD= Standard Deviation) for metals such as Fe, Co, Ni, Cu, Zn, Cd, and Pb were determined by digesting ten analytical reagent blank (n=10).

2.11 Analysis of the Selected Metals

Concentrations of Ca, Mg, Fe, Cd, Cu, Pb, Zn, Ni, and Co were analyzed using flame atomic absorption spectrometry; whereas analysis of Na and K was performed in flame photometer. Triplicate determinations were carried out for each metal and the mean values were reported. The instrumental conditions during the analysis of each the metal is present in Table 3.

Table 3: Standard conditions for Flame Atomic Absorption Spectrometry (FAAS) and flame photometry measurements

Metal	Na	K	Mg	Ca	Fe	Co	Ni	Cu	Zn	Cd	Pb
λ (nm)	589.0	766.5	285.2	422.7	248.3	240.7	232.0	324.7	213.9	228.8	283.3
Slit(nm)	0.2	4.0	0.4	0.4	0.2	0.2	0.2	0.4	0.4	0.4	0.4
I (mA)	5.0	5.0	2.0	3.0	4.0	4.0	4.0	3.0	3.0	2.0	2.0

NB: λ – wavelength, I - operating lamp current

3. Results and Discussion

3.1 Method Validation Parameters

The results of correlation coefficients are given in Table 4. According to (Mitra, 2003), the minimum correlation coefficient (r^2) should be 0.995. Thus, all calibration curves in this study showed good correlation between concentration and absorbance. The method detection limits determined for leaf and root bark samples were low enough to detect the presence of interested metals at trace levels (Table 4). The reproducibility of the analytical procedure was checked by carrying out triplicate analysis and calculating the relative standard deviation (RSD) for each metal. The recommended value of RSD is less than 10% (Mitra, 2003). In this study, RSD values were below 8% (Table 4) of the mean which revealed that the analytical method used was precise and reliable. As shown in Table 4, the percentage recovery varied from 83.3 to 112.9 %, which are in the acceptable range according to Clesceri *et al.* (1999).

Table 4: Correlation coefficient (r^2), Method detection limit (MDL) and recovery (R) results

	Na	K	Mg	Ca	Fe	Co	Ni	Cu	Zn	Cd	Pb
r^2	1.000	0.999	0.998	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.995
R (%)	104.2	83.3	106.7	112.9	88.5	91.7	86.4	105.2	85.4	88.8	95.0
MDL	-	-	-	-	0.09	0.03	0.03	0.002	0.005	0.01	0.08

NB: MDL in ppm, "-"denotes not analyzed

3.2 Concentration Level of Selected Metals in Leaf and Root bark of *Malva parviflora*

This study revealed that the leaf part contains high amounts of Na, K, Mg, and Ca. Similarly, high amounts of Mg and Ca were detected in the root bark. Appreciable amounts of Fe and Zn, and low amount of Cu were detected in both leaf part and root bark. Amounts of Co, Ni, Cd, and Pb were below the method detection limit (Table 5).

Table 5: Average concentration (ppm) ±SD of selected metals in leaf and root bark samples

Metal	Na	K	Mg	Ca	Fe	Cu	Zn	Ni	Cd	Pb	Co
Conc. in leaf part	1,875.0± 97.5	38,125.0 ±3,011.9	3,985.9 ±167.4	25,096.2 ±777.9	296.2 ±4.6	3.40 ±0.02	59.1 ±0.9	ND	ND	ND	NA
Conc. in root bark	NA	NA	3,614.4 ±133.7	13,427.1 ±590.8	891.2 ±14.3	5.45 ±0.04	197.5 ±2.8	ND	ND	ND	ND

NB: Conc. = concentration, ND=not detected, NA=not analyzed

The concentration level (38125.0±3011.9 ppm) of K indicates that leaf part of *Malva parviflora* rich in this element. K is the most abundant cation in the human body and its presence in edible part at high level is important (Ashraf *et al.*, 2010). A daily recommended amount of K for adult is 4,700 mg/day (Ismail *et al.*, 2011). K is a major component of many soils and derived from the weathering of soil parent materials such as potassium-aluminium-silicates. Other factors that increase amount of K in soils are application of fertilizers (Kirmani *et al.*, 2011) and plants and other organisms holding K as free ions in their cells, once they die; it quickly reenters the

soil solution (Grouh **and** Boroomand, 2012). The high amount of K in the leaf part may be attributed to the preferential uptake of K from the soil by *Malva parviflora*. This is further weak attachment of K to the soil particles which leads way for high leachability and absorption by plant roots (Grouh **and** Boroomand, 2012).

The concentration of Na detected in leaf sample was 1,875.0±97.5 ppm. The Na daily recommended range in developing countries is between 2400-5175 mg/day (Ismail *et al.*, 2011). The common salt which is used in cooking foods is the major source of Na uptake (Kirmani *et al.*, 2011). Therefore, *Malva parviflora* leaf is important alternative source of Na for patients who could not use table salt.

The concentrations of Mg detected in leaf and root bark samples were 3,985.9±167.4 and 3,614.4±133.7 ppm respectively. This result indicates *Malva parviflora* is a good source of Mg. The high content of Mg in this plant may be due to high absorption of Mg from the soil by the plant. Mg is widely distributed in animal foods and plants (Mohammed **and** Sharif, 2011). The overuse of fertilizers can accumulate high amount of Mg in top soils (Papastergios *et al.*, 2004). The daily recommended amount of Mg is 320-420 gm/day (Mohammed **and** Sharif, 2011). It plays an important role in protein synthesis (Ismail *et al.*, 2011), and its deficiency could be prevented by adequate consumption of leaf of Malva *parviflora*. The presence of Mg in medicinal plants enhances their antibacterial activity of against *Staphylococcus aureus* and *Escherichia coli* (Pandey and Karanwal, 2011) and actively promoting wound healing (Martiniakova *et al.*, 2009). Thus, its presence in *Malva parviflora* root bark might contributes wound healing and antibacterial activity of the plant.

The high amounts (25,096.2±777.9 and 13,427.1±590.8 ppm) of Ca were detected in *Malva parviflora* leaf and root bark respectively. In plants, leaf parts relatively contain high of Ca (Perveen *et al.*, 2012). It is one of the most abundant mineral in the human body (Selvaraju *et al.*, 2011), and the necessary daily intake is between 350 and 1,100 mg/day (Stef *et al.*, 2010). The high Ca content in the leaf of Malva *parviflora* suggests its possible use to overcome Ca deficiency. Ca also contributes antibacterial activity of medicinal plants against *Staphylococcus aureus* and *Escherichia coli* (Pandey and Karanwal, 2011) and promotes wound healing (Martiniakova *et al.*, 2009).

The concentration levels of Fe in leaf and root bark samples were 296.2±4.6 and 891.2±14.3 ppm respectively. The amount of Fe in leaf part was below the maximum permitted level (425 ppm) in vegetables (Uwah *et al.*, 2012). In this study, Fe was found high in leaf part, which is probably due to Fe-rich soil of the study area. Fe deficiency is one of the most prevalent nutritional deficiencies in the world (Ondo *et al.*, 2012). Therefore, use of *Malva parviflora* leaf in food preparation may be advised. The concentration of Fe in the root bark was in agreement with the amounts of Fe in selective medicinal herbs of Egypt (Maobe *et al.*, 2012). It is a key to wound healing wound healing (Martiniakova *et al.*, 2009) and enhances antibacterial activity of medicinal plants against *Staphylococcus aureus* and *Escherichia coli* (Pandey and Karanwal, 2011). Thus, the presence of Fe in root bark of *Malva parviflora* might contribute to its medicinal value.

The concentrations of Zn in leaf and root bark samples were 59.1 ±0.9 and 197.5 ±2.8 ppm respectively. According to Ismail *et al.* (2011), the concentration of Zn in leaf of *Malva parviflora* was below the maximum limit of Zn in vegetables (100 ppm). Besides, the range of Zn in agricultural products should be between 15 to 200 ppm (Jabeen *et al.*, 2010). About 20 % of the world's population could be at risk of Zn deficiency (Bhowmik *et al.*, 2012). The present study revealed that *Malva parviflora* leaf is a good source of Zn, and it can be advantage to overcome the Zn deficiency. It has also antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Pandey and Karanwal, 2011) and plays a vital role in wound healing (Martiniakova *et al.*, 2009).

The amounts of Cu in leaf and root bark samples were 3.40 ±0.02 and 5.45 ±0.04 ppm respectively. Its maximum limit in vegetables is 73 ppm (Shagal *et al.*, 2012). Permissible limits in medicinal plants for Cu set by China and Singapore were 20 ppm and 150 ppm respectively (Maobe *et al.*, 2012). The concentrations of Cu in both leaf and root bark of *Malva parviflora* were below limits. Most plants contain inadequate amount of Cu for their normal growth due its low mobility within plant tissues and uptake relative to other (Ondo *et al.*, 2012). Cu has also antibacterial activity against *Escherichia coli* (Varkey, 2010), and it promotes wound healing (Burns *et al.*, 2003).

Concentrations of Cd, Ni, Pb, and Co were not detected in this study. The failure to detection might due to their amounts were below the detection limits of the techniques used. Since Ni is essential micronutrient, its adequate supply for growing vegetables should be ensured through artificial or organic fertilizers (Itanna, 2002).

4. Conclusion

The present study revealed that leaf part of *Malva parviflora* contains high amounts of Na, K, Mg, and Ca. Similarly, high concentrations of Mg and Ca were detected in the root bark. Appreciable amounts of Fe and Zn, and low amount of Cu were detected in both leaf part and root bark. Concentrations of Co, Ni, Cd, and Pb were below the method detection limit. Since metals such as Na, K, Mg, Ca, Fe, Cu, and Zn play an important role to maintain a good human health, it's confirmed that the leaf of *Malva parviflora* is used for nutritional purposes. Besides, the results revealed that the leaf of *Malva parviflora* is a good source of these essential nutrients. The presence of high amount of Mg, Ca, Fe, Zn and Cu in the root bark might contribute to the therapeutic action of

Malva parviflora.

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