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Research Article

DETERMINATION OF ESSENTIAL AND NON-ESSENTIAL METALS CONCENTRATION IN PAPAYA (*CARICA PAPAYA*) SEEDS, LEAVES AND SUPPORTING SOIL OF ODO-SHAKISO DISTRICT IN SOUTH EAST OROMIA REGION, ETHIOPIA

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ABSTRACT

Traditional medicinal plants have been used in Ethiopia from ancient time to now and their modernization study focused on separation, identification and structural elucidation of active compounds. In this study; the levels of selected metals (Mg, K, Ca, Cr, Mn, Ni, Cu, Zn, Cd, Co and Pb) in papaya, one of the traditional medicinal plant and its supporting soil in Odo - Shakiso district of Guji Zone, Ethiopia, have been investigated. To analyse the above said nutrients, an efficient digestion procedure with optimized conditions was developed. The samples (papaya seeds, leaves and its supporting soil) were taken from three different sites namely Reji, Hawata and Dolobia of Odo-Shakiso district; digested with micro digestion techniques and selected metals analyses were made using Flame Atomic Absorption Spectrophotometer (FAAS). Even though, the level of some trace metals such as Zn found to be the highest in seeds sample while Mn found to be the highest in leaves sample; the concentrations were found to be bellow the maximum acceptable level as per FAO/WHO, different organizations and countries. In general, the nutritional (major, minor and toxic elements) analysis result of papaya seed and leaves suggested it to be a good source of major and minor elements; free from toxic metals.

Keywords: Medicinal Plant, Leaf, Seed, Microwave Digestion, FAAS, Carica Papaya Major, Minor.

INTRODUCTION

The contributions of medicinal plants since ancient times have been indispensable sources of both preventive and curative traditional medicine (TM) preparations for human beings and livestock [1]. Herbal medicines have a prominent role to play in the pharmaceutical and health markets of the 21 century [2]. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China, and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians. In Ethiopia the long history of using traditional medicinal plants for combating various ailments can be confirmed by referring to the medico-religious manuscripts in the country. The medicinal plant of Ethiopia and the developing countries play major supplementary roles to the limited modern health care available. The development of useful and widely used drugs like Digoxin and Digitoxin from Digitalis Leaves; quinine from the cinchona bark; reserpine from *Rauwolfia serpentine*; morphine from *Papaver somniferum*; coaine from *Erythroxzion coca* and the anti cancer *Vincristiner* and *Viblastine* from *Cartharathus troseus* of Madagascar and again anti-cancer compound, bruceatin from the Ethiopian plant, *Brucea antidysentrica*, just to name a few are examples of the contributions of traditional pharmacopoeia [3].

In Ethiopia, 70% of human and 90% of livestock population primary health care delivery are depend on traditional medicine similar to many developing countries particularly that of Sub-Saharan African countries [4]. According to the World Health Organization (WHO) report, almost 80% of people in marginal communities use only medicinal plants for the treatment of various diseases.

Among many traditional plants*, carica papaya* plants (**Figure 1 (a)**) has been studied under this research work. Papaya plant produces natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties [5]. The tea, prepared with the green papaya leaf **(Figure 1 (c)**), promotes digestion and aids in treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart [6]. The efficacy of treatments with *Carica papaya* is dependent on the quantity of the different compounds in the preparation. The quantity of the compounds, as previously indicated, differs in the fruit, latex, leafs, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree [5, 7]. Papaya leafs are also believed to be several health benefits including removing intestinal parasites, as aid in correcting digestion problems and can potentially reduce inflammation in several parts of the body [5].

The papaya seeds (**Figure 1 (b)**) are very pungent and peppery, making them almost unpalatable. Papaya seeds have antibacterial properties and are effective against E.coli, Salmonella and Staphylococcus infections; protect the kidneys from toxin-induced kidney failure; eliminate intestinal parasites and cure for piles and typhoid fever [8]. It is also reported that seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for impotence [9].

Papaya is considered one of the most important fruits because it is a rich source of antioxidant nutrients (e.g., carotenes, vitamin C, and flavonoids), the B vitamins (e.g., folate and pantothenic acid), minerals (e.g., potassium, magnesium etc), and fiber. In addition, papaya is a source of the digestive enzyme papain, which is used as an industrial ingredient in brewing, meat tenderizing, pharmaceuticals, beauty products, and cosmetics[10].

(a) (b) (C)

Fig. 1: Carica papaya; plant (a), (b) Seeds, (c) leaf in Odo-shakiso, Ethiopia

Different parts of *Carica papaya* is very important to cure a number of diseases and hence used by most of individuals in a regular basis in the country particularly in Guji Zone. For example *Carica papaya* leaves are best known to treat malaria. To treat malaria disease the leaves are boiled for a while and the extracted solution is inhaled from three to five days in an average. Powder of papaya seeds are extensively used to cure typhoid fever and to eliminate intestinal parasites (as dewormers). In addition young females used papaya seeds for illegal abortion. Even though different parts of *Carica papaya* are commonly used to treat a number of diseases, there is no any information about mineral contents in its different parts. The source of mineral nutrients for human being is plant materials consumed in the form of food or medicine. The human body requires both the metallic and the non-metallic elements within certain permissible limits for growth. All minerals are important for human body because they serve necessary functions[11, 12]. Even though some minerals required in very small amount, deficiency of trace elements cause diseases, whereas their presence in excess may result in disturbing normal functioning of organs and central nervous system. For example in human adults, peripheral nerve damage has been observed at 40 to 60 µg/dl lead contamination and anemia at 80 µg/dl[13]. High levels of lead exposure can result in stillbirth or miscarriage. It is one of the most toxic nonessential and mobile metallic elements found in soils and it affects animals and plant adversely [14]. Intake of cadmium-contaminated food causes acute gastrointestinal effects, such as vomiting and diarrhea [15]. Inhalation exposures to high levels of cadmium damage the respiratory system (bronchial and pulmonary irritation), headache, chest pains, muscular weakness, cancer and death[13].

Plants can easily be contaminated by heavy metals in the course of cultivation or later during the processing stage. The content of heavy metals is one of the criteria for the use of plant material as food or traditional medicines. Hence determination of mineral compositions in food and medicinal plant is essential for understanding their nutritive importance and health risk. Accordingly, control of heavy metals in medicinal plants and their products should be made such to ensure safety and efficacy of herbal products[4].

Carica Papaya seeds and leaves are widely used as traditional medicine in the world, having extensive commercial as well as social importance. However, no literature report was found on comparative determination of the concentration of essential and non-essential metals in papaya seeds, leaves. Owing to this, the present study reported comparatively the concentration of K, Mg, Ca, Mn, Cu, Zn, Co, Ni, Cr, Cd and Pb in papaya seed, leaves and supporting soil samples collected from Odo-Shakiso district using flame atomic absorption spectroscopy.

MATERIALS AND METHODS

Apparatus and Instruments

Ceramic pestle and mortar were used for grinding and homogenizing the C*arica papaya* leaves and seeds sample after sun drying at dust free place. All the *Carica papaya* seeds, leaves and soil sample were weighed on a digital analytical balance (ADAM, Model AFP-110L, England) with 120 g loading capacity and ± $1x10^{-4}$ precision. Microwave digester (Buck Scientific, Model BMS 1, and USA) was used for the digestion of *Carica papaya* seeds, leaves and supporting soil samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample till analysis. Flame atomic absorption spectrophotometer (Buck Scientific, Model 210VGP AAS, USA) equipped with deuterium background corrector and air-acetylene flame atomizer was used for the determination of concentrations of the metals (Mg, K, Ca, Cr, Mn, Ni, Cu, Zn,Co, Pb and Cd) in *Carica papaya* leaves, seeds and supporting soil samples.

CHEMICALS AND REAGENTS

Reagents that were used in the analysis are of analytical grade. HNO_3 (69-72 %) and H_2O_2 (30 %) (UNI- CHEM chemical reagent, China) were used for the digestion of *Carica papaya* seeds and leaves sample and HNO_3 (69-72 %) and HCl (37%) were used for supporting soil samples digestion. A solution of lanthanum nitrate [$La(NO_3).6H_2O$] was used to prevent the chemical interference in the determination of Ca and Mg, in the sample solution during the analysis. From the stock standard solutions containing 1000 mg/l of the metals Mg, K, Ca, Cr, Mn, Ni, Cu, Co, Zn, Pb and Cd (Buck Scientific Pure-Graphic[tm]), 10 mg/l of intermediate standard were prepared and used for the preparation of calibration standards of each metal. De-ionized water obtained from a water purification system (PUR1TE, D700 deionizer, France), and distilled water obtained from a water distillation (WWS/8 distillation apparatus, France) was used for cleaning, dilution and preparation of all solutions throughout the experiment.

Cleaning of Apparatus

Apparatus such as plastic kitchenware, crucibles, glassware, volumetric flasks, measuring cylinder, Teflon digestion vessels (DPA-60K) and other necessary materials used for the experiment were washed with detergents and then tap water followed by rinsing with de-ionized water. The apparatus were then soaked in 2 % nitric acid for one day and then rinsed with de-ionized water several times to make them acid free. Then the apparatus were dried in oven (Model N50C, England) and kept in dust free place until analysis began.

Description of the Study Area

Odo- Shakiso District is an administrative town of Guji Zone which is located in the South eastern of Oromia in Ethiopia. It is found at $6^0 14'$ N latitude and $38^0 10'$ East Longitude. It covers a total surface area of 187,150 Km^2. Odo-shakiso is bordered with Uraga District to North, Adola District to the East, Saba Boru District to the West. The capital town of the district is Shakiso, situated 405 km in the south from Addis Ababa on the way to Negelle. A map (**Figure 2)** and detail of the study area is given in **Table 1.**

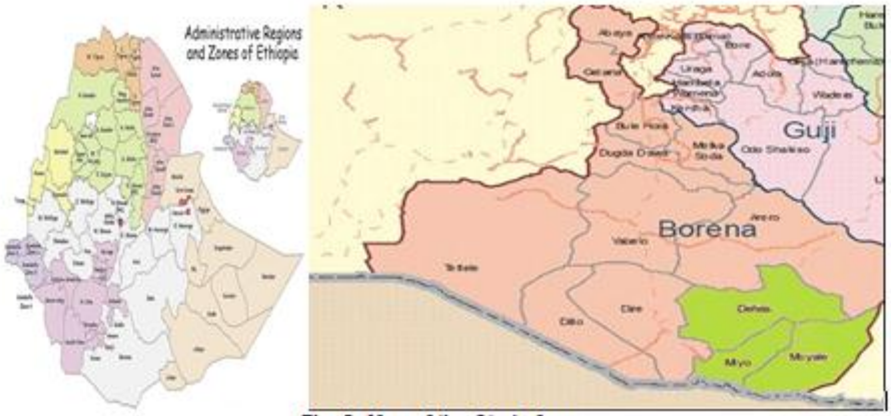

Fig. 2: Map of the Study Area

Table 1: Altitude, geographical location, annual mean temperature, and rainfall of the three sites

Kebeles	Latitude	Longitude	Altitude	Annual Mean Temperature	Annual mean rainfall
Reji	5⁰21'218""E	08⁰03'345"N	1650m	18 ⁰C - 25 ⁰C	1200 mm
Dolobia	5⁰20'447"E	08⁰07'689"N	1603m	16 ⁰C -28 ⁰C	1304mm
Hawata	5⁰195'04"E	08⁰09'670"N	1500m	17 ⁰C -30 ⁰C	1135mm

Sample Collection and Preparation

***Carica papaya* Seeds and leafs sample collection**

Representative amount of *Carica papaya* leaves and seeds sample were collected from Reji, Dolobia and Hawata sites in which people are taking these plant orally as a medicine. From each sites four sampling point were selected and from each sampling point 3 matured papaya plants were selected which means per-sites 12 matured papaya plants were taken. A total of 36 matured plants were selected from 3 sites and per plant 4 leaves were taken starting from the bottom to the tops. Papaya leaves collected from each sampling point was homogenized to get composite sample of each of the three sites. Similarly, Carica papaya seeds samples were taken from the same three sites with particular reference to the same plant one fruit from each papaya plant with a total of 36 fruit from three sites were collected. Then after 10 g from each plant and 30 g from each sampling point was carefully homogenized to get 120 g composite samples of each site with a total of 360 g from three sites *Carica papaya* seed samples were collected. The samples were packed into clean polyethylene plastic bags, labeled according to their sites and transported to laboratory for further treatment.

***Carica Papaya* leaves and Seeds Sample Preparation**

The leaves and seeds of the *Carica papaya* were separated from the plant with stainless steel teflon knifes, washed with a running tap water so as to remove adsorbed soil particulates and then rinsed with distilled de ionized water and air dried. A 300 g of the papaya leaves and 360 g of seeds were put on acid-washed labeled porcelain according to the samples sites and dried in oven at 60 ^{0}C for 24 hours till it got brittle and crisp. After cooling to ambient temperature, the dried samples were pound in to fine powder with mortar and pestle, and sieved with 1 mm sieve then which 50 g of leaves and seeds powder from each sites was used for analysis. The powdered samples were placed in pre-cleaned screw capped polyethylene container until digestion.

Soil Sampling and Sample Preparation

Soil samples were systematically collected from four different sampling points of each site at 75 cm canopy radius of the *Carica papaya* plant in 30 cm depth using stainless steel soil sampling auger. Since the investigation was concerned with possible uptake of essential and non-essential metals by *Carica papaya* plants, then the soil samples were collected from the whole area that the root system of the plant penetrates. The total amount of soil sample collected from three sites was 420 g providing that 140 g from each site, 35 g from each sampling point of which 50 g of sieved soil was used for chemical analysis. After removal of visible pieces of plant residues, the soil samples of each site were oven dried and homogenized. The dried soil samples were ground and sieved by using 1 mm nylon sieves. Before chemical analysis, the sieved soil sample was further dried in an oven at 50 ^{0}C for one and half hour to make its moisture content uniform. Finally the samples were stored in sealed polythene containers until analysis.

Optimization of the Working Procedure

The basic requirements of sample preparation for analysis are to get an optimum condition for digestion. In this study, to prepare clear and colorless sample solution which is suitable for analysis using FAAS, different *Carica papaya* seeds and leaves digestion procedures were optimized using HNO_3 and H_2O_2 acid mixtures by varying parameters such as reagent volume, microwave digestion temperature and time. Partly, Amare 2010[16], Kingston and Jassie 1986[17] optimization digestion techniques have been followed. The optimized procedures were selected based on; clarity of digests, minimal reagent volume consumption, minimal microwave digestion time, simplicity and temperature applied for complete digestion of samples. Optimized digestion procedures for *Carica papaya* seeds and leaves sample are shown in **Tables 2 and 3** respectively.

Table 2: Different trial tested during the optimization procedures for *Carica papaya* Leaves samples

No	Amount of sample (g)	Reagent volume (ml) HNO_3	Reagent volume (ml) H_2O_2	Observations
1	0.5	6	2	Clear and turbid
2	0.5	4	3	Clear but yellowish
3	0.5	4	4	Clear and turbid
4	0.5	5	2	Clear but yellowish
5	0.5	5	3	Clear and turbid
6	0.4	6	2	Clear and light yellow
7	0.4	4	3	Clear but yellowish
8	0.4	4	4	Clear but yellowish
9	0.4	5	2	Clear but yellowish
10	0.4	5	3	Clear and light yellow
1	0.2	8	2	Clear and colorless
2	0.2	7	2	Clear and turbid
3	0.2	6	2	Clear and Deep yellow
4	0.2	5	2	Clear but yello
5	0.2	4	2	Clear and light yellow
6	0.3	8	2	Clear and colorless
7*	**0.3**	**4**	**3**	**Clear and colorless**
8	0.3	3	2	Clear and Deep yellow
9	0.3	5	2	Clear and light yellow
10	0.3	4	2	Clear but light yellow

Microwave digestion program			
First Phase			
Steps	1'	2'	3'
Temperature (^{0}c)	180	210	50
Time (min)	15	10	5
Power (W)	80	90	40
*Second Phase			
Steps	1'	2'	3'
Temperature (^{0}c)	165	200	50
Time (min)	5	10	5
Power (W)	8	90	40

1 ' stands for initial conditions, **2'** stands for digestion conditions and **3'** stands for cooling conditions

From the 20 trials shown in **Table 2**, optimum conditions for Carica papaya leaves sample digestion are: a reagent mixture of 4 ml of HNO_3 and 3 ml H_2O_2**,** digestion temperature 200 ^{o}C, digestion time 25 minutes, power of 90 W and 0.3 g Carica papaya leaves (Trial number **7*,** second phase).

Table 3: Different trial tested during the optimization procedures for *Carica papaya* seeds sample

No	Amount of sample (g)	Reagent volume (ml)		Observations	Microwave digestion program			
		HNO_3	H_2O_2					
1	0.5	6	2	Clear and very light yellow	**First Phase**			
2	0.5	4	3	Clear and light yellow	Steps	1	2	3
3	0.5	4	4	Clear and very light yellow	Temperature ^{0}c)	185	215	50
4	0.5	5	2	Clear and light yellow	Time (min)	15	10	10
5	0.5	5	3	Clear and very light yellow	Power (W)	80	90	40
6	0.4	6	2	Clear and very light yellow				
7	0.4	4	3	Clear and light yellow				
8	0.4	4	4	Clear and very light yellow				
9	0.4	5	2	Clear and light yellow				
10	0.4	5	3	Clear and very light yellow				
1	0.2	7	1	Clear and very light yellow	*Second Phase			
2	0.2	7	3	Clear and colorless	Steps	1	2	3
3	0.2	6	3	Clear and turbid	Temperature ^{0}c)	175	200	50
4	0.2	5	3	Clear and very light yellow	Time (min)	5	15	10
5	0.2	4	3	Clear but yellowish	Power (W)	80	90	40
6	0.3	7	1	Clear and very light yellow				
7	0.3	5	5	Clear and colorless				
8*	**0.2**	**3**	**3**	**Clear and colorless**				
9	0.3	5	2	Clear and very light yellow				
10	0.3	4	3	Clear and light yellow				

1 ' stands for initial conditions, **2'** stands for digestion conditions and **3'** stands for cooling conditions

From the 20 trials shown in **Table 3**, optimum conditions for *Carica papaya* seeds sample digestion are: a reagent mixture of 3 ml of HNO_3 and 3 ml H_2O_2**,** digestion temperature 200ºC, digestion time 30 minutes, power of 90 W and 0.2 g *Carica papaya* seeds (Trial number **8*,** second phase**).**

Digestion of Samples

Many analytical methods including atomic absorption spectrometry for trace element determination in plant materials require decomposition of the sample. The common methods used for dissolving samples for metals analysis are acid digestion (wet-ashing), pressure-ashing, dry-ashing and microwave digestion. Of these analytical digestion methods microwave digestion procedure was used in this research work [18]. Microwave digestion is a rapid and efficient method for sample decomposition prior to the determination of trace metals [17, 18].

Digestion of *Carica papaya* Leaves and Seeds Sample

For the digestion of *Carica papaya* leaves and seeds samples, a mixture of conc. HNO_3 (69-72 %) and conc. H_2O_2 (30 %) were used. Powdered *Carica papaya* seeds samples (0.2 g) from each sites and each of the three dried powdered *Carica Papaya* leaves samples (0.3 g) from each sites were directly transferred in to DPA-60K Teflon digestion vessels followed by the addition of HNO_3 (3 ml) and H_2O_2 (3 ml) for *Carica papaya* seeds and HNO_3 (4ml) and H_2O_2 (3 ml) for *Carica papaya* leaves sample. The mixture was then shaken carefully until the solid material dissolved. The vessels were placed in a fume-hood for 15 min for pre-digestion and placed inside the turntable of the microwave system and heated in the microwave.

After completion of the program the power was put off and the digestion vessels kept in the fume hood. The digestion vessels were cooled to room temperature to avoid foaming and splashing. Then, the digestion vessels were opened carefully in a fume hood. The resulting clear and colorless solutions were cooled at room temperature and transferred quantitatively to 10 ml volumetric flasks and made up to the mark with de-ionized water. Each *Carica papaya* leaves and seeds samples were digested in triplicate. The digested and diluted sample solutions were then be stored in tightly capped polyethylene bottles and kept in refrigerator for further analysis by atomic absorption spectroscopy.

Digestion of the Supporting Soil Samples

The conventional aqua-regia digestion procedure consists of digesting soil samples is so widely used and European Community Bureau of Reference has certified several soil and sludge samples based on it [19]. In this work, for digestion of soil samples the EPA 3050B method was applied with a very slight modification. For the digestion of soil sample aqua-regia was prepared in the ratio of 2.5 ml (HNO_3): 7.5 ml (HCl) and added in a digestion vessel containing 0.5 g of the dried and sieved soil sample. The sample was digested at 200 ^{0}C for 25 minutes. Finally, after completion of digestion the digestate was allowed to cool, filtered through Whatman No. 42 filter paper and the resulting clear light yellow solution was made up to 25 ml with deionized water. Reagent blanks were also prepared and digested with the same procedure as that of soil sample. All the solutions were stored in tightly capped polyethylene bottles and stored in a refrigerator until analysis [20].

Digestion of the Blank Samples

Digestion of reagent blank was also performed in parallel with each of the seeds, leaves and soil samples keeping all digestion parameters the same. For the analysis of each seeds, leaves and soil samples six reagent blank were prepared. All the digested samples were stored in refrigerator until the analysis.

Instrument Operating Conditions and Calibration

Instrument Operating Conditions

In this study a total of eleven metals for each of seeds, leaves and supporting soil samples were analyzed using flame atomic absorption spectrophotometer with external calibration curve. For each metal three replicate determinations were carried out. Ten elements (Mg, K, Ca, Cr, Mn, Ni, Cu, Zn, Co, Cd and Pb) were determined by absorption mode while K was determined by emission mode. The operating conditions of the instrument for FAAS employed for each analytes are shown in **Table 4**.

Table 4: Instrumental operating conditions for determination of metals in *Carica papaya* seeds, leaves and soil samples using FAAS

Elements	Parameters				
	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Sample energy (ev)	Instrumental detection limit (mg/l)
Mg	285.2	0.7	1.0	3.717	0.001
K	766.5	0.7	2.0	--	0.010
Ca	422.7	0.7	2.0	3.912	0.010
Cr	357.9	0.7	2.0	2.712	0.04
Ni	341.5	0.2	7.0	2.624	0.020
Co	240.7	0.2	4.5	2.746	0.050
Cu	324.7	0.7	1.5	3.938	0.005
Zn	213.9	0.7	2.0	3.237	0.005
Mn	279.5	0.7	3.0	3.913	0.03
Pb	283.2	0.7	2.0	2.874	0.040
Cd	228.9	0.7	2.0	3.214	0.01

Instrument Calibration

Intermediate standard solutions of each metal containing 10 mg/l were prepared in 100 ml volumetric flask from the standard stock solutions that contained 1000 mg/l. The intermediate standards were diluted freshly with de-ionized water to obtain five working standards of each metal of interest for calibration purpose. The instrument was calibrated using five series of working standards. Concentrations of working standards and value of correlation coefficient obtained from Absorbance/transmittance verses concentration calibration curve for each metal are listed in **Table 5**.

Table 5: Concentrations of working standards and correlation coefficients for calibration

Metal	Concentration of working standards (mg/l)	Correlation coefficient (r)
Mg	0.01, 0.10, 0.30, 0.60, 1.0	0.9995
K	0.05, 1.00, 2.00, 3.00, 4.00	0.99956
Ca	0.20, 1.00, 2.00, 3.00, 4.00	0.99665
Cr	0.05, 0.50, 1.00, 2.00, 3.00	0.99966
Ni	0.06, 0.10, 0.30, 1.00, 2.00	0.9992
Co	0.06, 0.2, 0.40, 0.60, 1.0	0.99919
Cu	0.01, 0.10, 0.30,0. 60, 1.0	0.99991
Zn	0.01, 0.10, 0.50, 1.00, 2.00	0.99905
Mn	0.04, 0.15, 0.55, 1.00, 2.00	0.9995
Pb	0.05, 0.10, 0.20, 0.4, 1.00	0.99975
Cd	0.015, 0.10, 0.5, 1.00, 1.50	0.99903

Method Detection Limit (MDL)
Method detection limit is the minimum concentration of analyte that can be identified, measured and reported with 99 % confidence that the analyte concentration is greater than zero. In the present study, to know the method detection limit of each metal, six blank for each were digested and analyzed along with *Carica papaya seed*, leaves and supporting soil samples. Then the mean concentration of the blank and the standard deviation of the six blank samples were calculated for each metal. Finally, the detection limits were obtained by mean concentration of the blank plus three times of the standard deviation of the reagent blank. As shown in **Table 6**, the method detection limit of each element is above the instrument detection limit.

Table 6: Method detection limit for determination of metals in seeds, Leaves and soil samples

Metal	Instrumental detection limit (mg/l)	*MDL for soil (mg/l)	*MDL for leaves (mg/l)	*M DL for seeds (mg/l)
Mg	0.001	0.27	0.61	0.61
K	0.01	0.76	1.32	1.27
Ca	0.01	1.50	3.53	3.53
Cr	0.04	0.85	0.14	0.13
Mn	0.03	0.037	0.04	0.038
Ni	0.020	0.14	0.19	0.19
Co	0.005	0.72	0.094	0.091
Cu	0.005	0.06	0.07	0.066
Zn	0.005	0.34	1.24	1.22
Pb	0.04	0.06	0.08	0.072
Cd	0.01	0.07	0.02	0.08

*MDL = method detection limit

RESULTS AND DISCUSSION
Method Validation
Due to the absence of certified reference material for leaves and seeds sample in the laboratory, the efficiency of the optimized procedure was checked by adding known concentration of each metal in 0.3 g of *Carica papaya* leaves and 0.2 g seeds samples. The spiked and non-spiked samples were digested and analyzed in similar condition. Then the percentage recovery of the analyte was calculated by:

$$\text{Recovery} = \frac{\text{Cm in the spiked samples} - \text{Cm in the non-spiked samples}}{\text{Amount added}} \times 100\ \%$$

Where, Cm = concentration of metal of interest

As shown in **Table 7 and 8** the results of percentage recoveries for the studied metal nutrients in both *Carica papaya* seeds and leaves sample lie within the acceptable range 90-105 % and 90-109.2 % respectively. Therefore, this verifies that the optimized digestion procedure was valid (good accuracy) for *Carica papaya* leaves and seeds sample analysis.

Table 7: Recovery test for the optimized procedure of *Carica papaya* seed sample

Elements	[a] Conc. in sample (µg/g)	Amount added (µg/g)	[b] Conc. in spiked sample (µg/g)	Recovery (%)
Mg	4900 ± 1	980	5880.8 ± 2.1	96 ± 0.51
K	16978.3 ± 25.66	3300	20106.7 ± 3.4	94.8 ± 0.21
Ca	7680 ± 25	1500	9165 ± 1.09	99 ± 0.35
Cr	2.05 ± 0.13	1.5	3.54 ± 0.01	95.55 ± 0.5
Mn	23.3 ± 2.00	7.5	31.01 ± 0.02	102.74 ± 0.47
Ni	4. ± 0.05	1.5	5.43 ± 0.03	95.56 ± 045
Co	0.60 ± 0.04	0.9	1.43 ± 0.13	91.78 ± 0.37
Cu	`14.033 ± 1.1	4.5	18.36 ± 1.07	96.25 ± 0.88
Zn	75.4 ± 0.05	22.5	95.65 ± 0.4	90 ± 1.01
Pb	0.78 ± 0.01	0.9	1.65 ± 0.02	97 ± 0.65
Cd	0.71 ± 0.04	0.9	1.66 ± 0.01	105 ± 0.73

Table 8: Recovery test for the optimized procedure of *Carica papaya* leaves sample

Elements	[a] Conc. in sample (µg/g)	Amount added (µg/g)	[b] Conc. in spiked sample (µg/g)	Recovery (%)
Mg	4006.67 ± 10.41	800	4734.67 ± 0.43	91 ± 0.9
K	16041.70 ± 45.09	3200	19536.1 ± 6.6	109.2 ± 1.61
Ca	11345 ± 36.55	2250	13527.5 ± 0.8	97 ± 2.05
Cr	1.73 ± 0.21	1	2.68 ± 0.04	95 ± 0.08
Mn	30.41 ± 2.63	6	35.99 ± 0.43	93 ± 09
Ni	3.067 ± 0.03	1	4.12 ± 0.03	105 ± 0.44
Co	1.57 ± 0.07	1	2.49 ± 0.21	91.78 ± 0.8
Cu	16.33 ± 0.71	4	20.08 ± 0.62	93.75 ± 046
Zn	50.27 ± 0.03	10	60.6 ± 1.41	103.3 ± 1.03
Pb	0.69 ± 0.39	1	1.703 ± 0.02	101.3 ± 0.7
Cd	0.23 ± 0.35	1	1.21 ± 0.01	97.5 ± 0.06

[a]Mean concentration ± SD of samples analyzed in triplicate
[b]Mean concentration ± SD of spiked samples in triplicate

Determination of the Concentration of Selected Essential and Non-essential Metals in *Carica Papaya* Seeds, Leaves and Supporting Soil Samples of Each Sites

In the present study the concentration of nine essential (Ca, Mg, K, Zn, Cu, Mn, Co, Cr and Ni) and two non essential metals (Cd and Pb) in the *Carica papaya* leaves, seeds and soil samples of the three sampling sites (Dolobia, Reji & Hawata) were quantified by FAAS. Among the analyzed metals Ca, Mg, K, Zn, Cu and Mn were detected in all samples where as lead and Nickel was found to be below the method detection limit in leaves and seed sample but detected in supporting soil samples. The concentration of Cadmium, Chromium, and Cobalt was below the method detection limit in all samples. The low levels of the toxic metals might be an evidence for the absence of the use of some commercial fertilizers and herbicides for *Carica papaya* plantation in the three sites. The concentration values of the metals and their corresponding SD are shown in **Tables 9. 10, 11**.

It was checked with ANOVA at 95 % (p = 0.05) confidence level that for all quantified metals there is no significant difference in concentrations of the same metals from three sites.

Table 9: Average concentration (mean µg/g ± SD, n = 3) of metals in *Carica papaya* seed samples from the three sites

Elements	Dolobia	Reji	Hawata
Mg	4859.67 ± 54.02	4900 ± 1.02	4890.67 ± 48.25
K	16917.2 ± 26.92	16978.3 ± 25.66	16953.7 ± 19.05
Ca	7626.5 ± 28.55	7680 ± 25.00	7654 ± 27.51
Mn	21.8 ± 1.43	23.3 ± 2.00	22.42 ± 1.76
Cu	13.3 ± 1.2	`14.03 ± 1.11	13.7 ± 1.14
Zn	73.67 ± 0.76	75.4 ± 0.05	74.53 ± 0.91
Cr	ND	ND	ND
Ni	ND	ND	ND
Co	ND	ND	ND
Pb	ND	ND	ND
Cd	ND	ND	ND

ND = not detected

Table 10: Average concentration (mean µg/g ± SD, n = 3) of metals in *Carica papaya* leaves samples

Elements	Dolobia	Reji	Hawata
Mg	3959.17 ± 17.55	4006.67 ± 10.41	3981.2 ± 24.26
K	15946.7 ± 54.41	16041.70 ± 45.09	15971 ± 62.25
Ca	11278.7 ± 37	11345 ± 36.55	11321.7 ± 46.24
Mn	27.33 ± 2.33	30.41 ± 2.63	29.56 ± 3.17
Cu	15.11 ± 0.57	16.33 ± 0.71	16.03 ± 0.072
Zn	44.91 ± 1.86	50.27 ± 0.03	46.56 ± 1.39
Cr	ND	ND	ND
Ni	ND	ND	ND
Co	ND	ND	ND
Pb	ND	ND	ND
Cd	ND	ND	ND

Table 11: Average concentration (mean ± SD, n = 3 µg/g) of metals in Papaya root supporting soil samples

Elements	Dolobia	Reji	Hawata
Mg	914.83 ± 42.78	939.17 ± 29.54	925.83 ± 36.37
K	2300.17 ± 47	2382 ± 56.72	2342.67 ± 50.38
Ca	55.2 ± 10.3	57.33 ± 10.54	56.52 ± 2.91
Mn	942 ± 45.79	953.50 ± 36.79	944.166 ± 38.55
Ni	56.15 ± 0.79	59.27 ± 1.28	57.53 ± 1.50
Cu	35.46 ± 1.27	39.67 ± 1.64	36.18 ± 2.54
Zn	57 ± 2.78	60.97 ± 1.51	58.18 ± 2.78
Pb	12.97 ± 1.76	15.11 ± 0.85	13.82 ± 1.28
Co	ND	ND	ND
Cr	ND	ND	ND
Cd	ND	ND	ND

Comparison of the Concentration of Identified Metals using t-test

Numerically, concentrations of K, Mg and Zn are higher in papaya seed than papaya leaves (**Table 12**). Concentrations of K, Mg, Ca and Zn are higher in papaya leaves than supporting soil samples (**Table 14**). Cu and Mn higher in supporting soils than seeds **(Table 13)**. The statistical t-test result at 95% (p=0.05) were done for all and reveals that except Cu there is significant difference between metal concentration of leaves and seed samples of the same sites for all metals; there is significance difference between concentration of seeds and supporting soil samples of the same sites for all metals; there is significance difference between concentration of leaves and supporting soil samples of the same sites for all metals.

Table 12: Concentration of metals in papaya leaves and papaya seeds from each site

Metal	Sites					
	Dolobia		Reji		Hawata	
	Papaya seeds	Papaya leafs	Papaya seeds	Papaya leafs	Papaya seeds	Papaya leafs
Mg	4859.67 ± 54.02	3959.17 ± 17.55	4900 ± 1.02	4006.67 ± 10.41	4890.67±48.25	3981.2 ±24.26
K	16917.2 ± 26.92	15946.7 ± 54.41	16978.3±25.66	16041.70±45.09	16953.7±19.05	15971 ± 62.25
Ca	7626.5 ± 28.55	11278.7 ± 37	7680 ± 25	11345 ± 36.55	7654 ± 27.51	11321.7 ±6.24
Mn	21.8 ± 1.43	27.33 ± 2.33	23.3 ± 2.00	30.41 ± 2.63	22.42 ± 1.76	29.56 ± 3.17
Cu	13.3 ± 1.2	15.11 ± 0.57	`14.03 ± 1.11	16.33 ± 0.71	13.7 ± 1.14	16.03 ± 0.072
Zn	73.67 ± 0.76	44.91 ± 1.86	75.4 ± 0.05	50.27 ± 0.03	74.53 ± 0.91	46.56 ± 1.39
Cr	ND	ND	ND	ND	ND	ND
Ni	ND	ND	ND	ND	ND	ND
Co	ND	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND

Table 13: Concentration of metals in papaya Seeds and papaya Soils from each sites

Metal	Sites					
	Dolobia		Reji		Hawata	
	Papaya seeds	Supporting Soils	Papaya seeds	Supporting Soils	Papaya seeds	Supporting Soils
Mg	4859.67 ± 54.02	914.83 ± 42.78	4900 ± 1.02	939.17 ± 29.54	4890.67±48.25	925.83 ± 36.37
K	16917.2 ± 26.92	2300.17 ± 47	16978.3±25.66	2382 ± 56.72	16953.7±19.05	2342.67 ± 0.38
Ca	7626.5 ± 28.55	55.2 ± 10.3	7680 ± 25	57.33 ± 10.54	7654 ± 27.51	56.52 ± 2.91
Mn	21.8 ± 1.43	942 ± 45.79	23.3 ± 2.00	953.50 ± 36.79	22.42 ± 1.76	944.16 ±38.55
Cu	13.3 ± 1.2	35.46 ± 1.27	`14.03 ± 1.11	39.67 ± 1.64	13.7 ± 1.14	36.18 ± 2.54
Zn	73.67 ± 0.76	57 ± 2.78	75.4 ± 0.05	60.97 ± 1.51	74.53 ± 0.91	58.18 ± 2.78
Ni	ND	56.15 ± 0.79	ND	59.27 ± 1.28	ND	57.53 ± 1.50
Pb	ND	12.97 ± 1.76	ND	15.11 ± 0.85	ND	13.82 ± 1.28
Cr	ND	ND	ND	ND	ND	ND
Co	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND

Comparison of the Concentration of Metals in Papaya Leaves with Literature Values

Even if there is a difference in sample preparation and analysis techniques, the results obtained in the present study were compared with the values reported by different researchers in different medicinal plants (**Table 15**).

Comparison of Current Results with Maximum Permissible Limits (MPL) Set By Different Organizations and Countries

Literature information's were not found for maximum permissible limits of heavy metals in papaya seeds and leaves. Therefore comparison was made with standards set for other medicinal plants (**Table 16**). The levels of metals found in papaya seeds and leave samples were below the maximum permissible limit set for other medicinal plant according to the international standards for heavy metals. From this it can be inferred that currently there is no health risk associated with heavy metals during the consumption of papaya seeds and leaves from the three sites.

Pearson Correlation of Metals

To check associations of the same metal in soil with seed and leaves as well to check whether the ions of one kind present in the soil, either facilitate or interfere with the uptake of the other kind of ions, Pearson Correlation coefficient was used. A Pearson Correlation coefficient is a number between -1 and +1 that measures the degree of association between two variables (call them concentration of metal X and Y). A positive value for the correlation implies a positive association (large values of X tend to be associated with large values of Y and small values of X tend to be associated with small values of Y). A negative value for the correlation implies a negative or inverse association (large values of X tend to be small values of Y and vice versa) [24](Table 17).

Table 14: Concentration of metals in papaya leaves and supporting soil samples from each sites

Metal	Sites					
	Dolobia		Reji		Hawata	
	Papaya Leaves	Supporting Soils	Papaya Leaves	Supporting Soils	Papaya Leaves	Supporting Soils
Mg	3959.17 ± 17.55	914.83 ± 42.78	4006.67 ± 10.41	939.17 ± 29.54	925.83 ± 36.37	925.83 ± 36.37
K	15946.7 ± 54.41	2300.17 ± 47	16041.70±45.09	2382 ± 56.72	2342.67 ± 0.38	2342.67 ± 0.38
Ca	11278.7 ± 37	55.2 ± 10.3	11345 ± 36.55	57.33 ± 10.54	56.52 ± 2.91	56.52 ± 2.91
Mn	27.33 ± 2.33	942 ± 45.79	30.41 ± 2.63	953.50 ± 36.79	944.16 ±38.55	944.16 ±38.55
Cu	15.11 ± 0.57	35.46 ± 1.27	16.33 ± 0.71	39.67 ± 1.64	36.18 ± 2.54	36.18 ± 2.54
Zn	44.91 ± 1.86	57 ± 2.78	50.27 ± 0.03	60.97 ± 1.51	58.18 ± 2.78	58.18 ± 2.78
Ni	ND	56.15 ± 0.79	ND	59.27 ± 1.28	57.53 ± 1.50	13.82 ± 1.28
Pb	ND	12.97 ± 1.76	ND	15.11 ± 0.85	13.82 ± 1.28	57.53 ± 1.50
Cr	ND	ND	ND	ND	ND	ND
Co	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND

Table 15: Comparison for the quantified metals concentration (μg/g) in papaya leaves with other medicinal plants

Medicinal plant	Origin	Plant Part used	Concentration (μg/g) of metals in medicinal plants				
			Ca	Mg	Mn	Zn	References
Taraxacum officinale	Spain	leaves	29247	4461	101	68	21
Mentha piperita	Bulgaria	leaves	21131	5483	116	45	21
Calotropis procera Ait	Nigeria	Leaves	18900	-	231.5	71.7	5
Croton macrostachyus	Ethiopia	Leaves	7040	2961	420	61	16
Camellia sinensis	Thailand	Leaves	6550	2549	1512	55.40	5
Carica papaya	Ethiopia	Leaves	11345	4006.67	30.41	50.27	Present study

Table 16: Comparison of current results for papaya seed and leaves with maximum permissible limits set by FAO/WHO and different organizations and countries

Metals	Present study		MPL (µg/g)	Type of plant	The MPL is set by	References
	Papaya seed	Papaya leaves				
Mn	21.18±0.05	32.01±0.1	No MPL	Medicinal plants	WHO	22
Cu	12.81±0.02	15.42±0.07	20	Medicinal plants	China(2005)	23
Cr	ND	ND	2.0	Medicinal plants	Canada (2005)	5
Ni	ND	ND	1.5	Medicinal plants	No justified	22
Co	ND	ND	0.2	Medicinal plants	No justified	22
Cd	ND	ND	0.3	Medicinal plants	China(2005)	23

Table 17: Guidelines for interpreting strengths of positive or negative correlations (r) [24]

No	R	Strengths of correlations
1	> 0.70	Very strong positive relationship
2	0.40 to 0.69	Strong positive relationship
3	0.30 to 0.39	Moderate positive relationship
4	0.20 to 0.29	weak positive relationship
5	0.01 to 0.19	No or negligible relationship
6	-0.01 to -0.19	No or negligible relationship
7	-0.20 to -0.29	weak negative relationship
8	-0.30 to -0.39	Moderate negative relationship
9	-0.40 to -0.69	Strong negative relationship
10	> -0.70	Very strong negative relationship

Table 18: Pearson correlation coefficient for metals in soil samples with metal in seed

Elements	Mg in soil	K in soil	Ca in soil	Mn in soil	Zn in soil	Cu in soil
Mg in seed	0.866903	-0.47366	0.478739	0.13873	0.868182	0.304226
K in seed	0.037769	0.026295	-0. 866134	0.079676	0.183419	0.319153
Ca in seed	-0.02796	0.024409	0.006705	-0.06766	-0.1559	-0.27569
Mn in seed	-0.42111	0.96046	-0.44628	-0.0593	-0.29738	0.291095
Zn in seed	0.100313	0.562107	-0.24051	-0.32124	0.052489	0.35583
Cu in seed	-0.51142	0.363472	0.737039	0.842665	-0.12395	-0.36644

Associations of the same metals in soil samples with seed samples

As shown in **Table 18**, the more level of Mg in the soil, the more accumulation in papaya seeds. This verifies that the dependence of Mg concentration in the papaya seeds on the amount of metals under supporting soil of the plant. K in soil with K in seed, Ca in soil with Ca in seed, Mn in soil with Mn in seed and Zn in soil with Zn in seed have negligible relationship and moderate negative relationship between cu in soil with cu in seed samples.

Associations of metals of one kind in soil with metals of another kind in seed samples

As it can be seen from **Table 18** uptake of K concentrations by seed is strongly affected by Mg and Ca. In the other hand Ca in soil with Zn in seed and Mn in seed, K in soil with Mn in seed, K in soil with Zn in seed and Mg in soil with Mn in seed have negligible relationships. In similar trend one can interpret the relationship between the rests from **Table 18**.

Table 19: Pearson correlation coefficient for metals in papaya leave with soil samples

Elements	Mg in soil	K in soil	Ca in soil	Mn in soil	Zn in soil	Cu in soil
Mg in leaves	0.796442	-0.428622	-0.01506	-0.44742	0.073105	0.075658
K in leaves	0.132456	0.497685	-0.808636	0.870735	0.718606	0.639809
Ca in leaves	0.008292	-0.03327	-0.64827	-0.19424	-0.47587	0.092606
Mn in leaves	-0.0569	-0.01067	-0.37393	0.236693	-0.23311	0.244035
Zn in leaves	0.924369	0.612567	0.047484	-0.25064	0.096762	0.178831
Cu in leaves	0.856326	0.722866	-0.687396	0.136216	0.661503	0.293367

Associations of the same metals in soil samples with seed samples

From **Table 19**, the more level of Mg in the soil, the more accumulation in papaya leafs. This verifies that the dependence of Mg concentration in the papaya leaves on the amount of metals under supporting soil of the plant.

Associations of metals of one kind in soil with metals of another kind in leaves samples

As it can be seen from **Table 19** uptake of K concentrations by leaves is very strongly affected by Mg and Ca. In addition up take of Cu by leaf is strongly affected by concentrations of Ca in soils. This may suggest that, in papaya plants Cu absorption is influenced Ca metals in the supporting soil under papaya plantation.

CONCLUSIONS

The result of this study suggests that papaya seeds and leaves of the three sites contains appreciable amount of macronutrients and micronutrients. The order of metal concentrations (µg/g) in papaya leaves determined in this study is: K (15946.7 - 16041.7) > Ca (11278.7 - 11345) > Mg (3959.17 - 4006.67) > Zn (44.91 - 50.27) > Cu (15.11 - 16.33) > Mn (27.33 - 30.41). In the same way the order of metal concentrations (µg/g) in papaya seed determined is: K (16917.2 - 16978.3) > Ca (7626.5 - 7680) > Mg (4859.67 - 4900) > Zn (73.67 - 75.4) > Cu (13.3 - 14.03) > Mn (21.8 - 23.3). The results of this work showed that papaya leaves and seeds accumulate relatively higher amounts of K and Zn among the determined macro and micro nutrients respectively. But the order of metal concentrations in supporting soil determined in this study is slightly different from the order of metal concentrations (µg/g) in papaya seed and leaves samples determined i.e K (2300.17 - 2382) > Mn (942 - 953.5) > Mg (914.83 - 939.17) > Zn (57 - 60.97) > Ni (56.15 - 59.27) > Ca (55.2 - 57.33) > Cu (35.46 - 39.67) > Pb (12.97 - 15.11). The results of this work show that supporting soil samples contains relatively higher amounts of K and Mn among the determined macro and micronutrients respectively. The concentrations of detected metals in papaya leaves and seeds are within permissible limits set for metals in different medicinal plants by FAO/WHO, different organizations and countries. Seeds and leaves of Carica papaya cultivated in Odo-Shakiso district are free of toxic heavy metals like Pb and Cd.

Finally, the correlation between element pairs in papaya seeds, leaves and soil samples was statistically tested. The statistical analysis showed that, for most metals there was significantly strong positive correlation between the concentration of metals in papaya seed, leaves and supporting soil under papaya plantation. This may suggest that, in papaya plants metal absorption is controlled by the content of metals in the soil solution and bioavailability of metals in the supporting soil.

REFERENCES

1. Dery BB, Ofsynia R, Ngatigwa C. Indigenous knowledge of medicinal trees and setting priorities for their domestication in Shinyanga region, Tanzania. Nairobi, Kenya. International Center for Research in Agroforestry 1999.
2. Shad AK, Lajbar K, Iqbal H, Khan BM and Naveed A. Profile of heavy metals in selected medicinal plants. Pakistan Journal of Weed Science Research. 2008;14(1-2):101-110.
3. Desta B. Ethiopian traditional herbal drugs potentiality and appropriate utilization. In the proceedings of the 8th International Conference of Ethiopian Studies. 1988;1:763 - 765.
4. Endashaw B. Study on Actual Situation of Medicinal Plants in Ethiopia. Japan Association for International Collaboration of Agriculture and Forestry. 2007.
5. Ayoola PB and Adeyeye A. Phytochemical and nutrient evaluation

of Carica papaya (pawpaw) Leavesss. IJRRAS. 2010;5(3).
6. Mantok C. Multiple Usage of Green Papaya in Healing at Tao Garden. Tao Garden Health spa & Resort.Thailand.www.tao-garden.com. 2005.
7. Afolabi S, Gbenga D, Teminijesu O and Chizea V. Biochemical effect of some food processing methods on the health promoting properties of under-utilized Carica papaya seed. Journal of Natural Products. 2011;4:17-24.
8. Timothy Blalock. The Nutritional Value of Papaya Seeds. Asian Pacific Journal of Tropical Medicine: Seeds Nutrition Information.com. 2011;8(14): 26-31.
9. Elizabeth Kafaru. Immense help from nature,s workshop. Elikaf Health Services Ltd. Ikeja, Lagos, 1st ed. 1994;207-209.
10. Faostat: Crop Production. http://faostat.fao.org/ site/567/default.aspx#ancor 2012.
11. Soetan KO, Olaiya CO and Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: African Journal of Food Science. 2010;4(5):200-222.
12. Michael JG, Susan A and Aedin L. The Nutrition society textbook series, introduction to human nutrition, A John Wiley & Sons, Ltd., 2nd ed. 2009;203-251.
13. Peterso J, Donell M, Haroun L and Monette F. Radiological and chemical fact sheets to support health risk analyses for contaminated areas. Argonne National Laboratory Environmental Science Division. 2007;94.
14. Chaturvedi I. Phytotoxicity of cadmium and its effect on two genotypes of brassica Junceal. Emir. Journal of Agricultural Science. 2004;16 (2):01-08.
15. Godt J, Scheidig F, Brandenburg P, Reich A and Groneberg D. The toxicity of cadmium and resulting hazards for human health. Journal of Occupational Medical Toxicology. 2006;1:22: 1-6
16. Amare A. Metals in croton macrostachyus leaves and its infusion; A traditional medicinal plant in Ethiopia. Addis Ababa University 2010.
17. Kingston HM and Jassie LB. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Analytical Chemistry 1986; 58: 2534-2541
18. Kathryn J. L. and Steve J. H.: Microwave digestion procedures for environmental matrices, University of Plymouth, UK PL4 8AA, Analyst. 1998;123:103-133.
19. Chen M. Comparison of three aqua-regia digestion methods for twenty florida soils, Soil Science Society of America Journal. 2001;65: 491- 499.
20. Gaudino S, Galas C, Belli M, Barbizzi S, Zorzi P, Jacimovic R, Jeran Z, Pati A and Sanson U. The role of different soil sample digestion methods on trace elements analysis: a comparison of ICP-MS and INAA measurement results, Journal for Quality, Comparability and Reliability in Chemical Measurement. 2007;12:84 - 93.
21. Rashed M. Trace elements in some wild plants from the shores of the high dam lake and adjacent desert, as determined by Atomic Absorption Spectroscopy. Journal of Arid Environments. 1995; 29:185-197.
22. Khuda F, Iqbal Z, Zakiullah Z, Khan A, Nassir F, Muhammad N, Ali Khan J and Khan MS. Metal Analysis, Phytotoxic, Insecticidal and Cytotoxic Activities of Selected Medicinal Plants of Khyber Pakhtunkhwa. Pakistan Journal of Pharmacological Science. 2012;25(1):51-58.
23. Jabeen S, Shah MT, Khan S and Hayat MQ. Determination of Major and Trace Elements in Ten Important Folk Therapeutic Plants of Haripur Basin, Pakistan. Journal of Medicinal Plants Research. 2010;4(7):559 - 566.
24. Bolboaca SD and Jäntschi L. Pearson versus Spearman, Kendall's Tau Correlation Analysis on Structure-Activity Relationships of Biologic Active Compounds. Leonardo Journal of Sciences. 2006;9:179-180.