# Determination of Dietary Toxins in Selected Wild Edible Plants of Ethiopia

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# Abstract

Despite the great role of wild edible plants (WEPs) in ensuring food security and the fact that they can serve as a source of income, the consumption and acceptance varies from place to place. Although social, economic and cultural factors might have contributed for the avoidance of some edible wild plants, the fear of presence of toxic principles including heavy metals or their counter ions and antinutritional components might also be a barrier to the use of WEPs. In this study, the determination of the levels of some selected heavy metals (Cu, Cr, Ni, Cd and Pb) and antinutritional (oxalate) components are carried out using Flame Atomic Absorption Spectrophotometry and HPLC methods. The results showed that the mean metal concentration (mg/kg) in ripe fruit of WEP, unripe fruit of WEP and their underlying soil samples were 4.57, 5.77 and 3.22 (Cu), 2.37, 1.54, and 3.54 (Pb), 0.93, 0.63, and 0.42 (Cd), 2.02, 2.09, and 2.24 (Ni) and 2.47, 2.09, and 2.24 (Cr) respectively. The amounts of oxalate (mg/kg) in WEPs (Ripe Dovvalis Abyssinica, Unripe Dovvalis Abyssinica Unripe Ficus Sur Forresk and Unripe Blackberry) were 359.93, 301.01, 815.08 and 1406.15 mg/Kg respectively. All the heavy metals considered in this study and oxalate contents were detected in both WEPs and their underlying soil samples. The highest concentration of the heavy metals was found in blackberry while the lowest in unripe strawberry. Using one way ANOVA, significant (P = 0.05) variations among the levels of heavy metals in ripe and unripe were recorded. Generally, the content of heavy metals was higher in most of WEPs than their underlying soils. In conclusion, on the basis of the level of heavy metals and oxalate content, blackberry is found to be less safe for consumption than other WEPs considered in this study.

Keywords: Wild edible plants, heavy metals, anti-nutritional factors, oxalate, HPLC.

# Introduction

Wild edible plants (WEPs) refer to plant species that are neither cultivated nor domesticated, but are available from their wild natural habitat and used as sources of food [1]. They play an important role in food security because of their potential to provide food even in erratic rainfall years when arable crops fail. They have also great potential to serve as a source of income; thereby improve the standard of living conditions for local communities. Ethnobotanical and nutritional studies on WEPs in Ethiopia have reported that, Ethiopia is endowed with an important diversity of domesticated and wild plant species [2]. Nevertheless, their popularity and exploitation as source of food and income is very low in Ethiopia. In addition, the consumption and acceptance varies from place to place. Why? Questions such as this require scientific investigation on factors that can be part of the answer. Although social, economic and cultural factors might have contributed for the avoidance of some edible wild plants [3], the fear of presence of toxic principles might also be a barrier to the use of WEPs as a source of food and income. It is a valid fear because not all wild plants that seem appetizing are safe to eat. Even the safe wild edibles can be harmful at certain stages or conditions. For instance, some wild edible fruits are safe to eat when fully ripe but can have deleterious effect on human health if eaten before ripe. It is envisaged that such effects should be related to chemicals or their transformation in the plant cells. Therefore screening of wild edible plants at different stages of ripening for their toxic components is very important. Such studies can contribute to the attempts to barrier to use WEPs as source of food and income by providing safety information to consumers and food industries or small scale enterprises in the field to produce a value-added commercial food product in the form of fruit juice or jams.

This study thus focuses on the determination of dietary toxins in the edible parts of the selected wild edibles. More specifically, natural toxins such as oxalic acid and selected heavy metals including Cr, Pb, Cd, Cu and Ni are the target analytes. Top ranked four WEPs was identified through a preliminary reconnaissance survey in selected Districts at Hawassa University technology Village. Samples was systematically collected and authenticated by the National Herbarium Department of Plant Biology and Diversity Management, Addis Ababa University.

Determination of the levels of target chemicals was carried out using spectrophotometric and chromatographic methods. The detected levels was compared with the minimum permissible levels set by the international and national regulations to comment on the safety of the plants for use as food source or means to generate income.

# Materials and Methods

# **Chemicals and Reagents**

All chemicals used in this study were of analytical grade reagents. Hydrochloric acid (36-38%), 2N HCL, nitric acid (69-72%), oxalic acid, KH2PO4, HClO4, HPLC grade water, Acetonitrile, H3PO4, H2SO4, Stock standard solution (Buck Scientific purographics calibration standards, USA) of concentrations 1000 mg/L of the selected (Pb, Cd, Cr, Ni and Cu) heavy metals, from which intermediate 100 mg/L standards obtained, were used for preparation of the calibration standards of each metal. Deionized water was used for sample preparation, dilution and rinsing apparatus prior to analysis.

# Apparatus and Instruments

Polyethylene plastic bags were used to pack the harvested samples. Other instruments including drying oven (Digit heat, J.P. Selecta, Spain) or muffle furnace for ashing; ceramic mortar and pestle for grinding and homogenizing the samples; digital analytical balance (ADAM, Model AFP-110L, England) for weighing samples; pH meter used for measuring the pH of the WEPs and soil samples. Measuring cylinders, pipettes and micro pipettes, were used. Flame atomic absorption spectroscopy (Buck Scientific, Model 210 VGP AAS, USA) equipped with deuterium background corrector and air-acetylene flame atomizer was used for the determination of the heavy metals. An Agilent technology (1200 infinity series) HPLC equipped with UV detector was used for determination of oxalate content.

#### Sample collection

Two methods were involved a data collection through preliminary survey and Experimental methods. On the availability of the WEPs, *Dovyalis Abyssinica*, Ficus Sur Forresk, blackberry and strawberry representative amount of fruits were harvested, collected and packed into polyethylene plastic bags, labeled and transported to the chemistry research laboratory. The WEPs and their underlying soils were collected from three sites. Equal mass of WEPs and their underlying soils was taken from each site, and then it was homogenized. Total of four WEPs and their respective soils samples were collected. Soil samples (three replicates) were collected at the surface level (0 - 20 cm in depth) from locations where the WEPs samples were grown, harvested and collected.

#### Sample pretreatment

The Fruits of edible plants was collected manually; using vinyl gloves for protecting hands. The bruised portions were removed and the remaining samples were packed in the polyethylene bags for transporting to the Analytical Laboratory. In the laboratory, collected plant samples were washed with tap water and then with double distilled water to eliminate adsorbed dust and particulate matters. The plant samples were then cut and chopped into small pieces using plastic knife in order to facilitate drying. The samples were then air-dried for five to six days and further dried in a hot air oven at 60 °C for 24 h, to remove moisture and maintain constant mass. The dried samples were grinded into powder using commercial mortar and pestle and then sieved to mesh size.

# Sample preparation for physico-chemical analysis

For the determination of the physicochemical parameters including moisture content, total solids, ash content and pH in the soil and wild edible plants (AOAC, 2000) [4] method was used.

# **Extraction procedure of oxalate in WEPs**

Four gram of homogenized WEP sample was weighed and transferred in to 250 mL of shaking bottle. Fifty milliliter of 2N hydrochloric acid was added and mixed. The content of the bottle was shaked for 50 minutes in 250 rpm. The shaking bottle was then removed from the shaker and 50 mL HPLC grade water was added. Then appropriate volume of extract was filtered through 0.45  $\mu$ m syringe filter. Finally, the filtrate was transferred in to 2 mL vial and the vial was capped.

# **Digestion of Edible fruit parts of WEPs**

For each of the wild edible fruit, 1 g of powdered and homogenized samples was weighed and transferred to 250 mL of round bottom flask. To this, different volumes of HCl, HNO<sub>3</sub>, HClO<sub>4</sub>, at specified proportions (v/v) was added and digested at different temperatures 150, 200, 250, and 300 °C for different duration of time ( 60 min, 90 min, 120 min, and 180 min). The optimized procedure was determined based on the formation of clear solution. The digested solutions was allowed to cool and 5 mL of distilled-deionized water was added to dissolve the precipitate formed on cooling and gently swirled and filtered into 25 mL volumetric flask through Whatman filter paper. The clear solution then was diluted up to 25 ml with distilled-deionized water and stored for analysis by flame atomic absorption spectroscopy.

#### **Digestion of the soil samples**

One gram of crushed, powdered, sieved and homogenized soil samples was weighed and transferred to a 250 mL round bottom flask. To this 10 mL of 6:2:2 ratios of HNO<sub>3</sub>, HCl and HClO<sub>4</sub> were added. The solutions was then digested at 150 °C, 200 °C, 250 °C and 300 °C for 2 hrs. Then, the digested solutions were left to cool and 5 mL distilled-deionized water was added and gently swirled and filtered through Whatman filter paper. The filtrates was collected in a 25 mL volumetric flask was filled to the mark with distilled-deionized water and analyzed by flame atomic absorption spectroscopy.

#### Statistical analysis of data

The data obtained were analyzed by a computer program to analyze tabulated data using Microsoft Excel 2007 and origin 8 Software. One-way ANOVA (alpha = 0.05) was used to assess the statistical level of significance of the difference between and within the data obtained with samples from different sources (soil versus plant, ripe versus unripe etc). Correlation analysis and validation against Beer's law was made using least square liner regression model. The result P value was below 0.05 and this indicates the difference between the results of soil and plant, ripe and unripe WEPs was significantly different.

# **Results and Discussion**

# Physicochemical analysis of wild edible plants and their underlying soil samples

In this study, the physicochemical parameters including moisture content, total solids, ash content and pH were determined. For the determination of the physicochemical parameters in the soil and the wild edible plants the method of (AOAC, 2000) [4] was used. The results of physicochemical analyses (moisture content, total solids, ash content and pH) of the WEP samples and their underlying soils are summarized in **Table 1**.

The moisture content of the WEPs considered in this study ranged from 59.77 to 73.20 %, while the underlying soil of WEP samples ranged from 23.70 to 42.79 %. The moisture content of the wild edible plants is higher than their underlying soil samples. From the WEP samples moisture content of ripe blackberry was the lowest, while unripe *Dovyalis Abyssinica* was the highest. In addition the moisture content in the soil samples ranged from 23.70 to 42.79 %. The moisture content of the well edible plants is ranged from 23.70 to 42.79 %. The moisture content of the WEP samples in the current study was comparable with previously studied (*Ficus carica linnaus*, 30–70 %) [5]. Moisture content below 50 % is considered as lower moisture sample and above 50 % is considered as higher moisture samples. Thus the moisture content of the WEP samples is above 50 %. This indicates the fruits may be easily attacked by microbial and the constituents like oxalate inside the fruits may decompose in short period of time.

Ash content is the index of mineral contents in plants such as calcium, sodium, potassium, nickel and zinc [6]. In the current study the ash content of the WEPs was ranged from (1.2 to 9.0 %). The lowest ash content was recorded in unripe strawberry, while the highest in unripe *Dovyalis Abyssinica*. Having high ash content means presence of more minerals and metals in plant while small ash content refers presence of little minerals in the plant. Thus unripe Dovyalis Abyssinica has higher mineral contents than the other samples. The ash content of Jamun (*Eugenia jambolana*) was 2.17 % which was reported previously by Shahnawaz et al [7]. This was consistent with the present study.

As shown in **Table 1**, the pH of the wild edible plants was ranged 3.01 to 5.73, while in the underlying soils of WEP samples from 3.67 to 6.68. All the wild edible plants are in the pH of acidic range, this may indicate the presence other organic acids. The pH of the soil samples is also below pH = 7 i.e. in acidic range. As reported by Tamene and Seyoum [8], soil pH is one of the most major factors influencing mobility and adsorption of heavy metals in soils. As shown in **Table 1**. In the current study, the pH of most soil samples was below pH 5 then more metals was transferred from the soil to the wild edible plants.

The total solid content of the WEPs was in the range between 26.80 and 40.23 with the highest value in ripe blackberry and the lowest in unripe *Dovyalis Abyssinica*. The total solids in the underlying soils of WEPs were in the range from 57.21 to 76.30. Total solid content of nutritional values of three different varieties (Trounja, Lagou, and Gounda) in Bangladeshi local markets reported by Parvin et al [9], were (85.9-86.8%). High concentrations of dissolved solids can lead to unpleasant taste and can bind with toxic compounds and heavy metals. Therefore suspended solids and organic substances may be organic acids were found in ripe blackberry in greater amount. In general, all of the physicochemical parameters in the wild edible plants and their underlying soils have positive and negative impact on the quality of the wild edible plant samples.

#### **Concentration of Heavy Metals in the wild edible plants**

WPs may have greater concentration of the metal accumulated in their tissue. This can have impact not only on the productivity but also make the vegetables unsuitable for consumption. When we know that the underlying soil where the WEPs grow contain higher concentration of heavy metals we should be curious about its level in the WEP samples. Even though, the poor bioavailability of heavy metals in soil, the plants may have high ability to accumulate them in their different parts [10]. The average concentrations of heavy metals in the WEPs are presented in **Table 2.** Concentration of Nickel in the wild edible plants ranged from  $0.87 \pm 0.100$  to  $10.3 \pm 0.250$  mg/kg. Nickel was present in higher concentrations in unripe blackberry, while in moderate concentrations in other samples. The highest concentration of nickel  $(10.3 \pm 0.250 \text{ mg/kg})$  recorded in unripe blackberry was below the previous report (grape  $1.42 \pm 0.570 \text{ mg/l}$  and strawberry  $1.08 \pm 0.030 \text{ mg/l}$ ) [11]. The permissible limit of nickel in plants according to WHO standard is 10 mg/kg. The concentration of nickel in the wild edible plants is below the acceptable standard limit except for unripe blackberry.

The highest copper content was found in unripe *Ficus Sur Forresk* ( $8.32 \pm 0.112 \text{ mg/kg}$ ) while in unripe strawberry it was lowest in concentration ( $1.45 \pm 0.015 \text{ mg/kg}$ ). This was below the previous report ( $10.7 \pm 1.110 \text{ mg/kg}$ ) [12]. The standard limit of copper is (40 mg/kg) regulated by FAO/WHO. As shown in Table 4.2 that in all wild edible plants, copper concentration is less than permitted level, so they are suitable for consumption. The concentration of Chromium in wild edible plants ranged from  $1.70 \pm 0.037$  to  $3.85 \pm 0.150 \text{ mg/kg}$  for all the samples with ripe blackberry having the highest concentration. This was below the previous report [12] (bilberry 50.15 mg/kg and ash berry 13.1 mg/kg) from urban and suburban habitats. The concentrations of chromium (Cr) in all types of wild edible plants were above the maximum permissible value (1.30 mg/kg).

Cadmium is known as a principal toxic metal, since excessive cadmium exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects and cancer [13]. The WHO recommends maximum permissible levels in raw plant materials for cadmium is to 0.30 mg/kg (WHO, 1996). The highest concentration of cadmium was found in ripe blackberry  $(2.52 \pm 0.19 \text{ mg/kg})$ , while the lowest concentration was found in ripe *Ficus Sur Forresk* (0.092 ± 0.07 mg/kg). In the current study, the concentration of cadmium in *Ficus Sur Forresk* and strawberry below the study previously reported  $(0.47 \pm 0.013 \text{ mg/kg})$  [14]. The concentration of cadmium in the wild edible plants is above the acceptable limit except for unripe blackberry, strawberry and unripe *Ficus Sur Forresk*, so the wild edible plants which are found at the selected Districts of Hawassa University technology Village are not safe to eat. Eating these wild edible plants may cause disease.

Lead creates health disorders such as sleeplessness, tiredness, hearing and weight loss [15]. Ripe blackberry has higher lead accumulation  $(5.75 \pm 0.062 \text{ mg/kg})$  than the other wild edible plants, while lowest concentration was found in unripe *Ficus Sur Forresk* (0.45 ± 0.063 mg/kg) (**Table 2**). In the current study ripe blackberry has higher lead accumulation  $(5.75 \pm 0.062 \text{ mg/kg})$ , but this was lower than previously reported (Strawberry (fruit) 12.1 mg/kg) [16]. Except for ripe blackberry, unripe black berry and unripe *Dovyalis Abyssinica* the rest values were below the WHO permissible limit, 2.0 mg/kg set by WHO. As shown in **Table 2** it can be seen that the trend of occurrence of the heavy metal concentration in wild edible plant samples analyzed was in order of Cu > Ni > Cr>Pb> Cd. This trend suggests that wild edible plants have higher concentration of copper, Nickel and Chromium than lead and cadmium. According to the current study, the metal accumulation capacity of the wild edible plants was put in the ascending order of strawberry < *Ficus Sur Forresk < Dovyalis Abyssinica <* black berry. From these blackberry is most accumulator and straw berry is least accumulator.

#### **Concentration of Heavy Metals in the underlying soil of wild edible plants**

The most important pathway through which human exposed to the toxic metals are soil-plant-human (food chain) and soil-human (incidental soil ingestion). Out of the two soil-to-plants transfer is the key components of human exposure to metals [10]. The concentrations of heavy metals in the underlying soil of wild edible plant samples taken from different sites were presented in **Table 3** below.

In the soil sample, Cu concentration occurred in a range of  $2.87 \pm 0.025$  to  $3.40 \pm 0.025$  mg/kg. The highest level ( $3.40 \pm 0.025$  mg/kg) of Cu was found in the soil of *Dovyalis Abyssinica* and the soil of strawberry had the least level ( $2.87 \pm 0.025$  mg/kg) of Cu. The highest level of Cu recorded in soil of *Dovyalis Abyssinica* ( $3.40 \pm 0.025$  mg/kg) was below the previous report ( $155.5 \pm 7.50$  mg/kg) [17] and (7.36 mg/kg) [18]. The permissible limit for copper concentration in soil is 36 mg/kg, so the results in this study are below the acceptable limit set by WHO.

Concentration of cadmium in the soil samples ranged between  $0.30 \pm 0.002$  and  $0.67 \pm 0.029$  mg/kg). The highest concentration of cadmium in soil of *Ficus Sur Forresk* ( $0.67 \pm 0.029$  mg/kg) was lower than previous report ( $2.18 \pm 0.10$  mg/kg) [17]. The concentration of cadmium recorded all the soil samples was below the maximum permissible limit set by (WHO, 2007), which is 3 mg/kg.

Concentration of lead in soil samples considered in this study was recorded to range between  $1.60 \pm 0.375$  and  $5.05 \pm 0.080$  mg/kg. The highest concentration of lead was recorded in the underlying soil of blackberry sample ( $5.05 \pm 0.080$  mg/kg while the lowest was recorded in the underlying soil of *Ficus Sur Forresk* ( $1.60 \pm 0.375$  mg/kg). This was below the previous report (14.23 mg/kg [18]. In the soil samples considered in this study, the concentration of lead was recorded below the permissible limit set by source (WHO, 1996) which is 85 mg/kg.

In soil samples the maximum acceptable limit set by WHO for Ni is 35 mg/kg. The concentration of nickel in the soil samples considered in the current study ranged between  $0.17 \pm 0.040$  and  $3.20 \pm 0.087$  mg/kg. [19],

reported that the mean nickel concentration in farm soils was (0.167 mg/kg). This was lower than the concentration of nickel in the soil samples of the current study, while Mean Ni concentration  $(14.6 \pm 0.417 \ \mu g/g)$  in soils reported by Naser et al [14] was much higher than the current study. The concentration of nickel in the current soils considered in this study was below the acceptable limit set by WHO. The Cr contents in the soil samples were found between the range  $0.75 \pm 0.062$  and  $4.22 \pm 0.037$  mg/kg. The highest and lowest contents of Cr occurred in the soils of strawberry and *Dovyalis Abyssinica*, respectively. The range of concentration of chromium in soil reported previously (22.73 to 25.86 ppm) [20] was higher than the current concentration of chromium in the soil samples. The permissible limit of chromium in soil sample set by WHO is 100 mg/kg, and then the concentration of Cr in all the soil samples is below the standard limit set by WHO.

In this study all the five metals i.e. Cr, Cu, Ni, Pb and Cd were detected in all soil samples. The mean metal concentrations (mg/kg) in the underlying soil of WEP samples were: Cu (3.22), Pb (3.54), Cd (0.42), Ni (2.28) and Cr (2.24). Generally the mean concentration of the heavy in the soil samples in ascending order were Pb> Cu> Ni> Cr> Cd. Lead was found in highest concentration while the concentration of cadmium was the lowest. In general, the concentrations of the heavy metals considered in this study were below the recommended limit set by WHO.

#### Heavy metal transfer (TF) in edible parts of the wild edible plants

Calculating transfer factor helps us to know which wild edible plant accumulates more heavy metals and which one is least accumulator. Accumulation factor is the ratio of concentration of heavy metals in the plants to the concentration of their underlying soils [21]. The soil to plant transfer factor is presented in **Table 4** below.

The accumulation factor for Cr, Ni, Cd, and Cu greater than 1 in most of the studied wild edible plants indicates greater accumulation. Those metals that have a high transfer factor migrate to the edible part of the plant easier than do those with a low transfer factor; this is the reason that these metals reflect their high accumulation values in various wild edible plants to such a high ratio. The accumulation factor for Pb is less than one indicates high heavy metal concentration in soil in relation to levels in wild edible plants and therefore low uptake of heavy metals to wild edible plants [22].

#### Determination of Total oxalate by HPLC Method

#### Analytical and chromatographic conditions

The current study describes a HPLC method for the determination of oxalate. The developed method was validated in terms of linearity and LOD. The linearity and accuracy of the method were tested using spiked WEP samples. Known amounts of oxalate standard (5 ppm) were spiked to the WEP samples tested before for oxalate content, in order to obtain percentage of oxalate recovery. The percent of recovery for the WEP samples was 100.92%. LOD were found to be 0.007 ppm, indicating the sensitivity of the method. Chromatographic conditions were carefully optimized to get satisfactory resolution between analyte and solvent used. The optimized mobile phase was a mixture of acetonitrile, water and  $KH_2PO_4$  with a flow rate of 1.0 mL/ minute. The detection was made at wavelength of 210 nm and the selected detector was UV detector. The first step in the analysis was optimizing the mobile phase, flow rate (1 mL/min), injection volume (5  $\mu$ l, column and detector temperature (50 °C) and sequence of a sample. The chromatograms of the WEP samples are shown in **Figure 1**. The retention time oxalate standard solution was used to identify the peak corresponding to oxalate, and peak integration of the standard was 2.225. The retention time of the WEP samples was near to the retention time oxalate standard.

#### Quantitative determination of Total oxalate by HPLC Method

Wild edible plants are rich in several nutrients and rural people consumed frequently. However, the main problem related to the nutritional exploitation of these kinds of plants is the presence of anti-nutritional and toxic principles [23]. Oxalate is one of the anti-nutritional factors that affect human health when it is above the standard limit. High dietary oxalate intake influences mineral and trace metal absorption in humans and may lead to calcium oxalate stone formation due to the ability of oxalate to form insoluble complexes with divalent cations in the gastrointestinal tract thereby increasing risk of kidney stones. The severity of consuming unripe wild edible plants is higher than the ripe wild edible plants. Due to this reason, the total oxalate content of species ripe *Dovyalis Abyssinica*, unripe black berries, unripe *Dovyalis Abyssinica* and unripe *Ficus Sur Forresk* were measured using HPLC method. The total oxalate contents of the selected WEP fruits are presented in **Figure 2**.

The total oxalate contents of some selected WEPs included in this study in varied widely from 301.01 to 1406.15 mg/kg dry weight. The highest level of total oxalate was recorded in unripe blackberry (1406.15 mg/kg) and the lowest level was recorded in unripe *Dovyalis Abyssinica* (301.01 mg/kg. The levels of total oxalate in the four WEPs follows the order Unripe black berry > Unripe *Ficus Sur Forresk*> Ripe *Dovyalis Abyssinica* > Unripe *Dovyalis Abyssinica* fruit. Unripe black berries were found to contain highest content of total oxalate.

Oxalic acid was predominantly found in unripe stage of banana fruit (50% of total titrable acid) and the acid decreased gradually with ripening (about 60% of its original value). Thus, oxalic acid was suggested to be, contrary to vegetable plants, actively catabolized in ripening banana fruit, playing an important role in fruit repining [24]. Out of the four wild edible plants analyzed in this study, unripe black berry is categorized under Extensive amounts of total oxalate 201–4014 mg/100 g dry weight. The rest three samples are categorized under high oxalate concentration fruits containing 26–99 mg/100 g fresh weight.

In present study, the highest oxalate content was recorded in unripe blackberry (1406.15 mg/Kg). This was higher than previous report (*Smilax zeylanica* L 0.11  $\pm$  0.17 g/100 g and *Caryota urens* L. 0.01  $\pm$  0.005 g/100 g) [23]. The total oxalate content in unripe Dovyalis Abyssinica (301.01 mg/kg) and ripe *Dovyalis Abyssinica* (359.93 mg/kg) was below the previous report (kiwifruit, 554 mg/kg FW) [25] and (seed kernel 469.1  $\pm$  1.80 mg/kg dry weight) [26]. The concentration of the oxalate in all the four WEPs in the present study was lower than the previous report (8680 mg/kg) [27]. However, the levels of oxalate detected in all the investigated WEP samples were still categorized under high oxalate concentration fruits containing 26–99 mg/100 g fresh weight.

#### Conclusions

Determinations of selected heavy metals and antinutritional factor (oxalate) were successfully carried out using Flame Atomic Absorption Spectrophotometry and HPLC methods respectively. The level of oxalate content on all the selected WEP samples was higher than the acceptable limit. High dietary oxalate intake influences mineral and trace metal absorption in humans and may lead to calcium oxalate stone formation due to the ability of oxalate to form insoluble complexes with divalent cations in the gastrointestinal tract thereby increasing risk of kidney stones. The concentration of the heavy metals and in the ripe and unripe WEPs was not consistent. Heavy metal content varies with time of harvesting and stage of maturity of crops. Generally the contents of heavy metals in WEPs were higher than their underlying soils. The concentrations of all the heavy metals considered in this study were in accordance with the acceptable limit set by WHO, whereas the concentrations of heavy metals in WEPs were not consistent. Nickel and copper concentration in WEP samples was below the recommended limit set by WHO while the concentration of chromium and cadmium was above the recommended vale set WHO. Except for ripe blackberry, unripe black berry and unripe *Dovyalis Abyssinica* the concentration of lead on rest values were below the WHO permissible limit. This may be due to the reason that heavy metals may be accumulated by plants from soil through their root or foliar absorption from the atmosphere.

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Sample	Taste	Color	рН	Total	Moisture	Ash
1			1	solids	Content (%)	Content (%)
Ripe blackberry	Sweet	Red	4.34	40.23	59.77	2.70
Unripe blackberry	Sour	Light green	4.28	33.70	66.26	2.10
Ripe strawberry	Sweet	Red	4.21	27.73	72.27	1.90
Unripe strawberry	Sour	Green	4.86	30.86	69.14	1.20
Ripe Dovyalis Abyssinica	Light sour	Yellowish	3.36	30.65	69.35	8.00
Unripe Dovyalis Abyssinica	Sour	Green	3.01	26.80	73.20	9.00
Ripe Ficus Sur Forssk	Sweet	Red	5.38	38.42	61.58	5.40
Unripe Ficus Sur Forssk	Bitter	Green	5.73	27.43	72.57	4.20
Soil of black berry		Dark brown	6.68	67.90	32.10	
Soil of strawberry		Dark brown	4.99	76.30	23.70	
Soil of Dovyalis Abyssinica		Dark brown	4.52	59.02	40.98	
Soil of Ficus Sur Forssk		Dark brown	3.67	57.21	42.79	

 Table 1. Results of physicochemical analyses of the studied WEPs\* and underlying soils

\*WEPs: Wild Edible Plants

Sample	Pb	Cd	Ni	Cr	Cu
Ripe blackberry	$5.75 \pm 0.062$	$2.52 \pm 0.190$	$2.50 \pm 0.140$	$3.85 \pm 0.150$	$6.45 \pm 0.123$
Ripe strawberry	$1.52 \pm 0.137$	$0.20 \pm 0.060$	$2.47\pm0.100$	$1.72 \pm 0.037$	$1.60 \pm 0.015$
Ripe Dovyalis A.	$1.50\pm0.080$	$0.90\pm0.010$	$0.87\pm0.100$	$2.43 \pm 0.150$	$4.72\pm0.050$
Ripe Ficus Sur F.	$0.70\pm0.042$	$0.09\pm0.070$	$2.22 \pm 0.075$	$1.87\pm0.030$	$5.52 \pm 0.125$
Unripe blackberry	$2.25 \pm 0.029$	$1.15 \pm 0.090$	$10.3 \pm 0.250$	$2.00\pm0.075$	$8.07\pm0.012$
Unripe strawberry	$0.57\pm0.052$	$0.17\pm0.010$	$1.37\pm0.087$	$1.70 \pm 0.037$	$1.45 \pm 0.015$
Unripe Dovyalis A.	$2.87\pm0.404$	$1.02 \pm 0.040$	$2.47 \pm 0.225$	$2.17 \pm 0.117$	$5.25\pm0.050$
Unripe Ficus Sur F.	$0.45 \pm 0.063$	$0.17 \pm 0.050$	$0.97 \pm 0.162$	$2.50 \pm 0.112$	$8.32 \pm 0.112$

Table 2. Metal means concentration (Mean ± stdev in mg/kg) in WEPs

Table 3. Metal means concentration (Mean ± stdev in mg/kg) in underlying soil of WEPs

Sample	Pb	Cd	Ni	Cr	Cu
Soil of blackberry	$5.05\pm0.080$	$0.30 \pm 0.002$	2.75	$2.50 \pm 0.005$	$3.23 \pm 0.062$
			±0.175		
Soil of Dovyalis A.	$3.25 \pm 0.029$	$0.37 \pm 0.110$	0.17 ±	$0.75 \pm 0.062$	$3.40 \pm 0.025$
			0.040		
Soil of Ficus Sur F.	$1.60 \pm 0.375$	$0.67 \pm 0.029$	3.00 ±	$1.50 \pm 0.050$	$3.37 \pm 0.042$
			0.375		
Soil of Strawberry	$4.25 \pm 0.125$	$0.32 \pm 0.105$	3.20 ±	$4.22 \pm 0.037$	$2.87 \pm 0.025$
			0.087		

Table 4. Transfer factors of heavy	metal from	soils into t	he WEPs.
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Sample	Pb	Cd	Ni	Cr	Cu
Ripe blackberry	$1.14\pm0.042$	$8.42\pm0.637$	$0.91\pm0.077$	$1.54\pm0.060$	$2.00\pm0.054$
Ripe Dovyalis A.	$0.46\pm0.025$	$2.45\pm0.730$	$5.00 \pm 1.278$	$3.23\pm0.350$	$1.39\pm0.020$
Ripe Ficus Sur F.	$0.44 \pm 0.11$	$0.14\pm0.013$	$0.74\pm0.096$	$1.25\pm0.050$	$1.64\pm0.040$
Unripe blackberry	$0.45\pm0.01$	$3.83\pm0.300$	$3.73\pm0.250$	$0.80\pm0.030$	$2.50\pm0.050$
Unripe Dovyalis A.	$0.88\pm0.125$	$2.79\pm0.800$	$14.1 \pm 3.500$	$2.90\pm0.290$	$1.54\pm0.024$
Unripe Ficus Sur F.	$0.28\pm0.04$	$0.26\pm0.080$	$0.33\pm0.070$	$1.67\pm0.090$	$2.47\pm0.050$
Ripe strawberry	$0.36 \pm 0.043$	$0.62\pm0.288$	$0.773\pm0.04$	$0.41\pm0.01$	$0.56\pm0.010$
Unripe strawberry	$0.14 \pm 0.013$	$0.54 \pm 0.17$	$0.429 \pm 0.03$	$0.41 \pm 0.01$	$0.51 \pm 0.010$



**Figure 1.** HPLC chromatogram of (A) Ripe *Dovyalis Abyssinica*, (B) Unripe blackberry (C), Unripe *Dovyalis Abyssinica* and (D) Unripe *Ficus Sur Forresk*.



**Figure 2.** HPLC results of oxalate content (mg.kg<sup>-1</sup>) of the wild edible plants RDA (Ripe *Dovyalis Abyssinica*), UBB (Unripe BlackBerry), UDA (Unripe *Dovyalis Abyssinica*), and UFSF (Unripe *Ficus Sur Forresk*).