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Determination of the level of some selected metals and nutritional value of white and redanchote tuber

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Abstract

White and red Coccinia Abyssinica (anchotes) are most popular food commonly used in Ethiopia, especially in western part of Oromia. This paper reports the level of some metals found in white and red anchote tuber and their nutritional value. Samples of white and red anchote were collected from Liban Jawi District, Oromia and then digested. The contents of the metals in the digests were analyzed using flame atomic absorption spectrometer (FAAS). Furthermore, proximate and nutritional composition of both varieties of anchote were determined. The concentration ranges recorded in unprocessed edible parts of white Coccinia Abyssinica (in mg/100g) samples were, Na (25.66), K (416.66), Ca (113.20) and Mg (13.5), and that of red Coccinia Abyssinica (in mg/Kg) in anchote sample were Na (570), K (510), Ca (101.80) and Mg (29.7). In red Coccinia Abyssinica, the concentration of K was the highest followed by Ca, Na and Mg while in white Coccinia Abyssinica, the concentration of K was the highest followed by Ca, Mg and Na. The proximate and nutritional composition value of red and white anchote were described as; moisture contents (78.13, 74.39) , crude protein (6.70 , 4.95), crude fat (0.35 ,1.75), crude fiber (5.05 ,4.76), total ash (1.04 , 1.46) and total carbohydrate (8.73 , 12.69) were recorded. The result shows that red anchote was higher in crude fiber, moitsure and crude protein content compared to the white anchote. However white anchote contained more amount of fat, ash and carbohydrate. Further investigation on other parameters and heavy metals are valuable to clearly understand the complete nutritional potential and safety of the crop.

Keywords: Essential metals, FAAS, Nutritional composition, Anchote tuber

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Introduction

Anchote (Coccinia abyssinica) is an indigenous tuber crop possessing variations in tissue colour which is available in Ethiopian Highlands, particularly popular in the western Oromia region of Ethiopia.

Coccinia Abyssinica (Anchote) tubers classified into two based on variations in its tissue color; these are red and white anchote (Yambo *et al*., 2013). White tissue anchote (*Coccinia Abyssinica)* appears to be more popular, as a result of its soft texture and

ease of cooking. However, the red variant was well thought-out for flour making (by dehydration), for use in breakfast cereal and soups for various medicinal and supplementary food applications. Red anchote (*Coccinia Abyssinica)* was have an important higher level of crude protein (p value = 0.041), with an average of 16.85 and 9.72 mg/100 g (on a dry matter basis) for red and white accessions respectively. White anchote shows a more important source of Ca with 81 mg/100 g edible portion; however, on dry matter basis, the content was similar to the red variant (316 and 309 mg/100 g dry matter, white and red respectively) (Parmar *et al*., 2017).

Approximately 4% to 6% of human body weight is composed of mineral elements, which are essential in the diet (Khan *et al*., 2014). Each of essential mineral nutrients has their own role for a proper body function and health status (WHO, 2004). Mineral contents such as calcium, magnesium, potassium and sodium are an important aspect of a balanced diet. Calcium for relaxing the central nervous system; magnesium to prevent muscle spasms; potassium and sodium for electrolyte balance (Millikan, 2012).

The mineral elements are mostly cofactors of many enzymes and thus have very important role in several physiological functions of humans and other animals(Khan *et al*., 2014; Stawarz *et al*., 2007). Each mineral nutrient has own role for a proper body function and health status (WHO, 2004). The deficiency of minerals would be severe health problems such as anemia, rickets, osteoporosis and diseases of the immune system (Frontela *et al.*, 2011; Norhaizan *et al*., 2009). The mineral contents of anchote (*Coccinia Abyssinica)* tuber samples were analyzed in previously, however, the study didn't identify these essential elements from *Coccinia Abyssinica* based on variation in their tissue color (Ayalew *et al*., 2016). In similar way, the proximate composition values of anchote (*Coccinia Abyssinica)* were determined but, didn't identify the proximate composition values based on variation in tissue color. Though white and red anchote (*Coccinia Abyssinica)* is economically, medicinally and socially significant plant and may contribute its part towards the goals of the country poverty improvement, very limited information is available in relation to its mineral content, proximate composition and economic value (Fekadu *et al*., 2014).

Until today, only few papers have been reported on white and red *Coccinia Abyssinica.* For instance, (Parmar *et al*., 2017) reported nutritional macro, micro nutrients levels such as Ca, Mg, P, K, Na, S,

Cu, Co, Fe, Mn, Se, and Zn in white and red anchote accessions. Empirical data is important to design and implement the intervention required for policy makers and other stakeholders, such as research system, rural and agricultural development system, and other government and non-government organizations. Without having such information, the possible intervention could not be effective. In view of this fact, this study was conducted to determine the proximate composition value and metal elements contents in white and red anchote (*Coccinia Abyssinica)* tubers sample taken from Liban Jawi district in Western Shoa, Oromia, Ethiopia. Therefore, the concentrations of four essential metals namely, calcium (Ca), sodium(Na), potassium(K), and magnesium(Mg) and six proximate compositions of white and red anchote were analyzed. The study is expected to provide useful information for future studies, about the white and redanchote (*Coccinia abyssinica)* crops, proximate composition, and minerals concentrations in its tubers.

Materials and methods

Description of Sampling Area

West Shoa Zone is located in Oromia region of Ethiopia having 22 districts. Anchote is the major staple, co-staple food used for many purpose and commercially available crop in the zone. For this study Babich market which is found in LibanJawi district were selected as sampling sites. Babich market is 50 Km and 161 Km away from Ambo and Addis Ababa respectively. The reason for selection of sampling sites was based on the proximity, maximum productivity, availability of the sample, transport accessibility and sampling cost. The study sites have sub-humid agro-ecology.

Materials and apparatus

White and red anchote samples were collected from market of Babich district west Shoa, Oromia. Polyethylene plastic bags were used for holding the white and red anchote samples. Chopping board (PTFE, China) and Teflon knife were used to cut the white and red anchote tubers into small pieces. Drying oven (DHG-9070A, Shanghai, China) was used for drying the white and red anchote samples. Porcelain mortar and pestle were used during grinding of the white and red anchote samples. Porcelain crucibles were used to dry the samples in oven.0.5 mm and 2.0 mm mesh sieve were used to sieve the ground white and red anchote samples. Electronic analytical balance (AA-200DS Deriver instrument company, Germany) was used for weighting the samples. Muffle furnace (Gallen kamp, size 3) was used to heat the

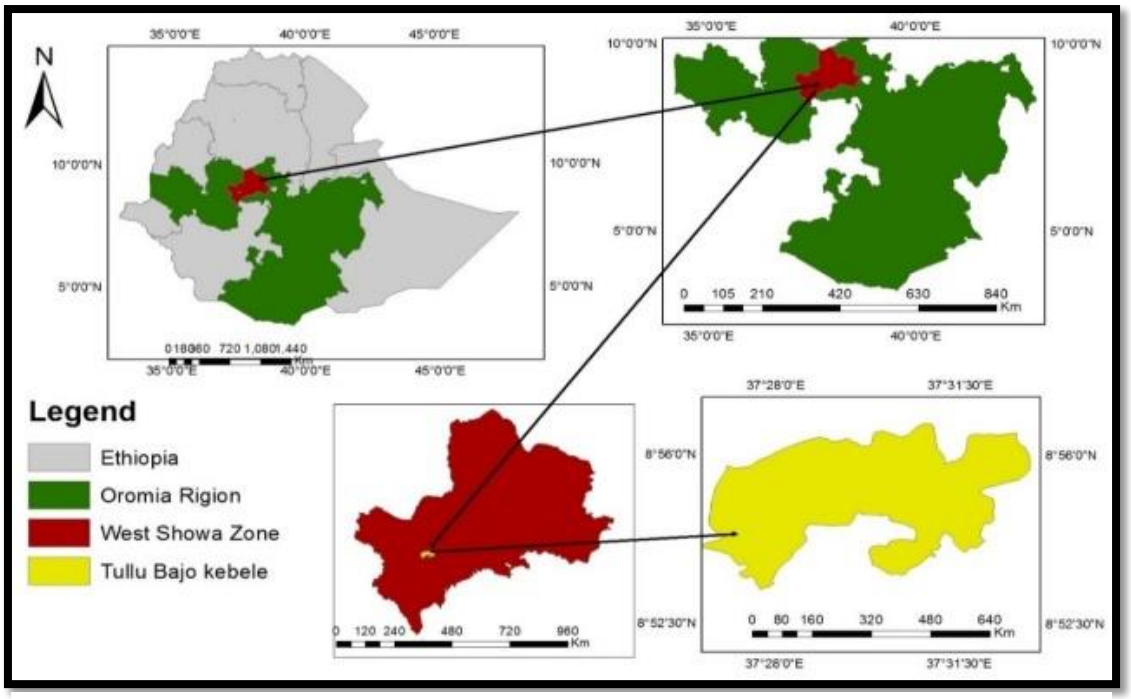

Figure 1. Location of the study area, source: (EthioGIS, 2015)

sample; Burate (50 mL) and conical flask (500 mL) were used for titration purposes in the determination of total nitrogen contents in samples to estimate crude protein. Digestive furnace (KDN-20 ^{0}C, China) was used to digest the dried and powdered white and red anchote samples. Borosilicate volumetric flasks (50,100 and 1000 mL) were used during dilution of sample and preparation of metal standard solutions. Measuring cylinders (5, 10, 25, 50, and 100 mL) and micropipettes (10-100 µL and 100-1000 µL) were used during measuring different volumes of samples solution, acid reagents and metal standard solutions. The digested samples were filtered using What man filter paper No.42. Capped glass bottles (100 mL) were used for storage of final digested samples. Refrigerator (Beko RDP 6900, Japan) was used to keep the samples ready for analysis till determination. Flame atomic absorption spectrophotometer (Model, AA-320N, Shanghai, China) equipped with deuterium back ground corrector and hollow cathode lamps with air/acetylene flame atomizer was used for the analysis of Ca and Mg flame photometer (ELICO CL-378, India) was used for analysis of Na and K.

Reagents and Chemicals

All chemicals and reagents used were of analytical grade. Concentrated HNO_3 (69 - 70%, Uni-Chem chemical reagents India), concentrated $HClO_4$ (69 -72%, Uni-Chem chemical reagents India), and Concentrated H_2O_2 (35% Uni-Chem chemical reagents India) were used for digestion of white and red anchote samples. Solution of 0.1%

$LaCl_3 \cdot 7H_2O$ (99.9%, Uni-Chem chemical reagents India.) was used prevent the chemical interference on Ca and Mg during the analysis of white and red samples. Potassium hydroxide (KOH), $C_{12}H_{23}O_{11}$ (99%, HIMEDI, AR) were used as a matrix for the method blank for the plant. Stock standard solution of concentration 1000 ppm of the metals K, Na, Ca and Mg standard solutions KCl(LOBA Chem PVT, Ltd, India), NaCl (99%,Uni-Chem,India),$CaCl_2.2H_2O$ (UNI-Chem, india) and $MgCl_2.6H_2O$ (98%,Uni-Chem, India) were used to prepare stock standard solution and intermediate standard solutions. Distilled water (chemically pure with conductivity 2.0µS/cm and below were used for dilution and preparation of reagents and standards solution as well as for rinsing glassware and sample bottles. Sodium hydroxide (NaOH), Hydrochloric acid (HCl) and Boric acid (H_3BO_3) were used. Universal indicator and phenolphthalein indicator solutions 3 g of catalyst were prepared from 10g of copper sulfate ($CuSO_4$) and 150 g of potassium sulfate (K_2SO_4) and used for digesting samples during determination of crude protein.

Roots and tuber samples collection and preparation

Tubers sample of white and red anchote (*Coccinia Abyssinica)* were collected from five sellers from market randomly in Liban Jawi District. Fresh samples of anchote were purchased from farmers in the market and collected in five containers. Since it is difficult to separate red and white tissue anchote from its external appearance, a small portion of the tubers skin was removed by cork, to identify underlying red and white parenchyma tissue visually, one-one composite samples (2 Kg) was made for both white and red anchote by observing and identifying its underlying color from five containers of Babich Market.

Figure 2. Variety of anchote a) anchote tuber before peeled; b) red anchote; c) white anchote

Determination of proximate values from white and redanchote

Determination of moisture

Moisture content was determined according to (AOAC, 1988), using the official method 925.09. Crucibles made of aluminum will be washed and dried in drying oven and allowed to cool in desiccators. The mass of each dried crucibles were taken first (M_1), and about 5 g of sample was weighed in clean and dried crucible (M_2) using analytical balance (Adventurer, OHAUS, China). The crucibles containing the samples were then put in an oven set at 105^0C to dry the sample to constant weight (M_3). Finally, moisture content was calculated using equation 4.

$$\text{Moisture (\%)} = \frac{M2-M3}{M2-M1} \times 100 \quad \text{------- (4)}$$

Where M_1 = Mass of the crucibl, M_2 = Mass of the crucible and the sample before drying, M_3 = Mass of the crucible and the sample after drying.

Determination crude protein

Crude protein was determined by Kjeldahl method according to (AOAC, 2000) using the official method 979.09. About 0.5g of sample was digested by heating with 6 mL of concentrated sulphuric acid (H_2SO_4), and mix with 3.5 mL of 30 % hydrogen peroxide solution. 3g of catalyst mixture prepared from 10g of copper sulfate (CuSO4) and 10g of potassium sulfate (K_2SO_4) was added to the digestion flask and digested at 370 ^{0}C for 4 h, in nitrogen determination apparatus until a clear solution was obtained. The digested samples were transferred into the fume hood for cooling and the content in each flask was diluted with distilled water and then neutralize with 35 % sodium hydroxide (NaOH) to make the solution slightly alkaline. Finally, samples were distilled and ammonia received in flasks containing excess 2 % boric acid (H_3BO_3) solution for reaction with ammonia. The reacted solution of ammonia borate was then titrated with 0.1N hydrochloric acid (HCl), to determine the total nitrogen.

$$\text{Nitrogen (\%)} = \frac{Nx(V2-V1)\times 14.007}{W\ X1000} \times 100 \text{ - (5)}$$

Where, V1 = Volume (mL) standard HCl solution in the titration of the blank, V2 = Volume (mL) standard HCl solution in the sample, W = Sample weight, N = Normality of HCl solution, 14.0070 = Molecular weight of Nitrogen.

NH_3 (ammonia) + H_3BO_3 (boric acid) → NH_4^+ + $H_2BO_3^-$ (borate ion)

The dilution, distillation and titration steps of the digested sample were conducted by using Kejeltic analyzer unit. The Kjeldahl method does not measure the protein content directly, a *conversion factor (F)* is needed to convert the measured nitrogen concentration to a protein

concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition.

Protein (%) = 6.25× % Nitrogen ------ (6)

Determination of crude fat

Ether extract method was used to determine the crude fat using soxhlet extraction apparatus by the official method 4.5.01(AOAC, 2000). moisture free sample (2 g) was weighed, in to each of the extraction thimbles wrapped with two centimeters layer of fat free cotton. Cleaned and dried receiving beakers were first weighed and filled with 70 mL of diethyl ether and fitted into the soxhlet apparatus for the extraction process. After 4 h of extraction, the ether in the receiving beakers were allowed to evaporate in a drying oven at 92^0C for at least 30 minutes, and then was cooled inside desiccators.

The percent crude fat content was determined by using the following formula from the Kjeldahl method to (AOAC, 2000).

$$\text{Crude Fat (\%)} = \frac{M2-M1}{W} \times 100 \text{-------- (7)}$$

Where, M_1= mass of dried beaker, M_2 = mass of beaker and lipid extract, W = Sample weight.

Determination of crude fiber

Crude fiber content of the samples was determined according to (AOAC, 2000), using the official method 962.09. Extraction, filtration, drying and combustion were involved in the steps for extraction process, 1.5 g sample was measured into each of 600 mL beakers, and 200 mL of 1.25 % H_2SO_4 was added and the contents were boiled for 30 min using hot plate. During boiling the level of the sample solution was kept constant with hot distilled water and periodically stirred. After 30 minutes 20 mL of 28 % potassium hydroxide (KOH) solution was added and further boiling for another additional 30 min following the above procedure. After completing the extraction process the sample solution was transferred to sintered glass crucible covered with 10 mm sand layer wetted with distilled water and then filter under vacuum. During filtration, the beaker was rinsed several times with hot distilled water and transferred into crucible. The sample residues were washed with 1% H_2SO_4 with successive rinsing using hot distilled water, followed by addition of the same volume of 1% NaOH solution with consecutive washing by hot distilled water. Once again, 1% H_2SO_4 solution was added with continuous addition of hot distilled water. At last, the residues were washed with water free acetone. Each of the crucibles with the respective contents was

dried in an electric oven for 2h at about 130^0C and cooled in desiccators before the weight was taken (M1). The dried residues in each crucible was transferred in to muffle furnace for 30 minutes' ignition at 550^0C and allowed to cool in desiccators before taking the final mass of each crucible (M_2). Finally, the crude fiber content was calculated using equation 8 below:

$$\text{Crude Fiber (\%)} = \frac{M2-M1}{W} \times 100 \text{----- (8)}$$

Where, M_1 = mass of the crucible and sample after drying in an oven, M_2 = mass of the crucible and sample after washing, W = Sample weight.

Determination of Total Ash

Total ash content was determined according to (AOAC, 2000), using the official method 923.03. The crucibles were first cleaned and dried in an oven at 100^0C and cooled in desiccators before the mass of each crucible were weighed by analytical balance (M_1). By taking 2.5g, sample (M_2) the crucibles were thoroughly charred on hot plate starting from low temperature under a hood, and then placed in a muffle furnace at about 550^0C until the sample was changed to grayish white ash which took about 5h. To take the final mass (M_3) the crucibles that contained-ignited sample was cooled inside desiccators. Finally, the total ash content was calculated by equation 9:

$$\text{Total ash (\%)} = \frac{M3-M1}{M2-M1} \times 100 \; -- \; (9)$$

Where, M_1 = mass of the dried dish, M_2 = mass of dish and the sample before ashing M_3 = mass of the crucible and the sample after drying.

Determination of carbohydrates

The utilizable carbohydrate content of the respective samples was determined using following formula (10):

Carbohydrate (%) = 100 – (% moisture +CF +CF +CP +Ash) ---------- (10)

Where, CF = crude fat, CF = Crude fiber, CP = crude protein

Analysis of Samples

Digestion of white and redanchote samples

The digestion condition was applied for white and redanchote samples. 0.5g of each of white and redanchote samples were weighted with mixtures of 2 mL of concentrated $HClO_4$, 5 mL of concentrated HNO_3 and 1 mL of H_2O_2.The mixture was digested on KDN-20 digestive furnace by setting the temperature at 240^0C for 3 h. The digestion was carried out in triplicate for each white and redanchote sample. Digestion of reagent blank also performed with the same procedure in parallel with digestion samples keeping all digestion parameters the same. Three blanks were digested for white and redanchote samples. The digested solution was allowed to cool to room temperature for 10 min without dismantling the condenser from the flask. To cool the solution 20 mL of de-ionized

water was added and the solution shacked to dissolve precipitates remained on the flask. This solution was transferred to 50 ml volumetric flask by filtering using what man No.42 filter paper and rinsing the round bottom flask with de-ionized water. Lanthanum chloride (1%) was added to prevent the chemical interference on Sodium, Potassium, calcium and magnesium and the solution was filled to the mark (50 %) with de-ionized water. These digested samples of bothanchote types were labeled and kept in the refrigerator at 4^0C until the determination of the level of the metals by FAAS.

Preparation of standards

Preparation of stock standard solution

Apparatus such as digestion flask tube, glassware, plastic containers, and polyethylene bags were washed with tap water using detergent followed by deionized water rinsing. The apparatus was then soaked in 10% HNO3 for 24h followed by rinsing with deionized water.Stock standard solution containing 1000 mg/L of the metals Na, K, Ca, and Mg were prepared from high purity metals and reagent grade salts using distilled water, nitric acid and Hydrochloric acid based on the individual analysis sheets for specific instruction of the instruments. The stock solutions were prepared at concentration of 1000 mg/L of the metals.

Calibration of standard preparation.

All working solution was prepared by diluting stock standard solution (1000mg/L) of the metals to be analyzed. The calibration standard solutions were used to calibrate the instrument response with respect to analyte concentration (USEPA, 2001). In this work, a series of four standard solutions which were lying within the working linear ranges of the instrument were prepared by serial dilution of the stock standard solution into 100 mL volumetric flasks. The prepared metal concentrations include; for K ,0.8, 1.4, 2.4 ,3.2 ,4 for Na, 1, 2, 3, 4, 5 for Mg, 1,2,3,4,5 and for Ca, 4,8,12,16,20.

Spiking metal standard mixture solution

A practical approach is to spike the concentration to reach near the mid-range of calibration point (Zheng *et al*., 2007). For the spiking process of the white and redanchote tubers samples; Standard mixture solution containing 50 mg/L of each metal was prepared. This mixture of standard solution was obtained by taking 5mL of K, Mg, Ca and Na from stock standard solution (1000 mg/L) into 50ml volumetric flasks and diluted to the mark with distilled water. The volume spike stock solution that should added to the

samples is calculated according to the equation (11).

$C_1V_1 = C_2V_2$---------- (11)

Where C_1 and C_2 are the concentration of the spike stock solution or intermediate and the desired spike concentration respectively, $V_1$and V_2 is volume of the sample to be spiked, and Volume of stock solution to be calculated respectively (Csuros *et al*., 2016; Zheng *et al*., 2007).

Method validation and quality control

Method validation

The criteria used for evaluating analytical methods are called figures of merits. Based on these characteristics; one can predict whether a method meets the needs of intended purpose. These figures of merit are accuracy, precision, sensitivity, detection limits and quantification limits (Mitra, 2003).

Precision and accuracy: In this study the laboratory precision of the results was assessed by the analysis of laboratory control sample (LCS) samples. To evaluate the analytical method accuracy and repeatability were determined using sample spikes. For doing, so, each samples was spiked in replicates of three at near mid-range calibration concentration (5mg/L for Na, K, Mg and Ca). The spiked samples were digested and analyzed following the same procedure as the samples. Precision was expressed as relative standard deviation (RSD) of replicate results. The RSD is calculated by dividing the standard deviation by mean value of the analytical data according to the following equation (Mitra, 2003).

$$\%RSD = \frac{S}{\bar{X}} \times 100$$---------- (12)

In this study, the accuracy of the analytical procedure was evaluated by doing recovery test. This was done by spiking a suitable known quantity of metal standard solution into a test portion of the sample. This includes analysis of the matrix spikes, laboratory control samples and methods blanks. The percent recoveries of the analyte were calculated using equation (13).

$$\% \text{ Recovery} = \frac{\text{CM the spiked sample} - \text{CM in the non-spiked sample}}{\text{CM added for spiking}} x100$$

---------- **(**13)

Where, CM = concentration of metal of interest

Instrument Detection Limit (IDL): IDL is the smallest signal above background noise that an instrument can detect reliably. The IDL is calculated to be the concentration equal to three times the standard deviation of three replicate measurements of calibration blank (USEPA, 2001).

$IDL = 3 \times S_{blank}$ ---------- (14)

In the present study instrument detection limit for each metal was determined from analysis of three replicates of calibration

blank and the results were calculated using equation (14).

Method Detection Limit (MDL): Method detection limit is the minimum concentration of a substance that can be measured and reported with 95% confidence that the analyte concentration is greater than zero. MDL was based up on three replicate measurements of a series of spiked blanks that are carried through the entire sample preparation scheme (USEPA, 2001).

The MDL was calculated by multiplying the standard of the three replicate measurements by the appropriate student's test value according to

MDL = t x s---------- (15)

Where, t = 3.14 (students" t" value for a 95% confidence level for three replicate or two degrees of freedom, S = standard deviation of the replicate analysis with n-1 degrees of freedom.

In this study two method blanks were spiked with three times the estimated IDL and MDL for each metal was estimated by digesting three replicates of the method blanks with the procedure for white and redanchote. Analyses of two blank samples for all metals of interest were performed and the standard deviation of the two blank reagents was calculated and the MDL was calculated by using equation (15).

Limit of Quantification: The lowest concentration level at which a measurement is quantitatively meaning full is called the limit of quantification (LOQ) (Mitra, 2003). In this study LOQ was obtained from triplicate of analysis of two reagents blanks which were digested in the same digestion procedure as actual samples. The LOQ was calculated by multiplying standard deviation of the reagents blank by ten plus the mean of the reagent blank signal (Miller *et al*., 2010). LOQ was calculated using equation (16).

LOQ = Mean blank +10$S_{blank,}$ ------- (16)

Quality control

Quality control is a series of analytical measurement used to assess the quality of the analytical data each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with analyte (s) of interest to evaluate and document data quality and analysis of standards and blanks as tests of continued performance (USEPA, 2001).

Contamination Control: Many measurement processes are prone to contamination, which can occur at any point in the sampling; sample preparation, sample collection, transportation, storage or analysis. Contamination becomes a major

issue in trace analysis. The lower concentration, the more pronounced is the effect of contamination. Contamination can occur in the laboratory at any stage of sample preparation and analysis. It can come from containers and reagents or from the ambient environment itself. In general, contamination can be reduced by avoiding manual sample handling and by reducing the number of discrete processing steps (Mitra, 2003).

Blanks: Blanks are samples that do not contain any (or a negligible amount of) analyte. Blanks are used to assess the degree of contamination in any step of the measurement process. They are made to stimulate the sample matrix as closely as possible. Blank samples from the laboratory and the field are required to cover all the possible sources of contamination. Different types of blanks were used, depending on the procedure and measurements objectives (Mitra, 2003).In this study $LnCl_3.7H_2O$ (99.1%) was used as matrix foranchote samples analysis.

Calibration Blank: Calibration blank is used to measure the amount of the analytical signal that arises from the solvents and reagents, and the zero solution in calibration series (Mitra, 2003).

Method Blank: Method blank is an analyte-free matrix to which all reagents are added in the same volumes or proportion as used in sample processing. The method blank should be carried through the complete sample preparation and analytical procedure. The method blank should be carried through the complete sample preparation and analytical procedure. The method blank is used to evaluate contamination that may occur during sample preparation and analysis. These could arise from the reagents the glass ware or the laboratory environment (Mitra, 2003).

Laboratory Control Sample (LCS): The LCS was analyzed in an identical manner as a sample and the results were used to document accuracy and precision of the analytical methodology. Result accepted within $\pm$15 of the truevalue (Csuros, 2016). In this study, 0.5g of Sucrose, with 2mL of spiked mixture of standards spiked in five replicates to white and redanchote samples, so, that the spike level was 4mg/L of each Ca, Mg, K, and Na were prepared in the laboratory and digested like the sample including exposure to all glass ware, digestion media, apparatus, solvents and reagents that are used with other samples. The percent LCS recoveries for each metal of interest were calculated using equation (17) (USEPA, 2001).

$$\%R = \frac{LCS}{S} x100 \text{ ---------- (17)}$$

Where % R = percent recovery, LCS = Laboratory Control Sample results, S = amount of spike added.

Instrumental calibration

Calibration of an instrument involves making of a comparison of a measured quantity against reference values. In this study, atomic absorption spectroscopy stock standard solution containing (1000 mg/L) of the metals were used for preparing intermediate calibration standard solutions(10mg/L) in 100 mL volumetric flask. The working standards at different concentration for each metal of interest (K, Na, Mg and Ca) were prepared using deionized water. The instrument was calibrated with working standards after adjusting the parameters (slit width, lamp current, wave length, and others) to give maximum signal intensity. The calibration graphs and correlation coefficients of each of the elements were determined by plotting working standard concentrations versus the corresponding absorbance. The level of four (Na, K, Mg and Ca) elements were determined by FAAS. Calibration curve was plotted for each of the metals standard using standard concentration against absorbance. Immediately after calibration, the sample solutions were aspirated into the instrument and direct reading of the metal concentration was recorded. Three replicates determinations were carried out on each sample. The same procedure was employed in the determination of elements in all digested blank.

Elemental analysis of samples

The concentration of Ca, Mg, K and Na in the samples was calculated by using equation (18). Concentration (mg/Kg) = $\frac{Concentration\left(\frac{mg}{L}\right)}{M} \times V$---------- (18)

Where: V = final volume of solution after digestion, M = initial weight of sample measure.

Statistical analysis

One –way analysis of variance (ANOVA) and Microsoft Excel Software will be employed at 95% confidence level to assess existence or absence of significant difference in mean concentration among different samples at ($p > 0.05$). ANOVA can be used in situations where there is more than one sources of random variation (Miller, 2005).

Results and discussion

Analysis of proximate composition

The results of proximate and nutritional compositions were present in both red and white anchote in Table 1. Protein, moisture and crude fiber contents were highest in the

red anchote when compared to white anchote. While Carbohydrate, Fat and Ash contents were highest in white anchote.The mean concentration and standard deviation of proximate composition value of red and white anchote were described as shown in Table 1.

Table 1. Mean, SD, RSD of nutritional and proximate composition value per 100 g (raw, peeled, unprepared, eatable portion) of red and white anchote at p=0.05 value.

parameters	Red anchote			White anchote		
	Mean	SD	RSD	Mean	SD	RSD
Moisture Content (%)	78.13	1.49	1.91	74.39	0.82	1.10
Crude Protein (%)	6.70	0.25	3.73	4.95	0.15	3.03
Crude Fat (%)	0.35	0.006	1.71	1.76	0.06	3.40
Crude Fiber (%)	5.05	0.06	1.19	4.76	0.08	1.68
Total Ash (%	1.04	0.02	1.92	1.46	0.09	6.16
Utilizable Carbohydrate (%)	8.73	0.40	4.58	12.69	0.36	2.83

STD = standard deviation, RSD = Relative standard deviation

Table 2. Mean (±SD) Proximate composition value per 100 g (raw, peeled, unprepared, edible portion) of red and white Coccinia abyssinica at p=0.05 value

Treatment	Moisture Content (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Total Ash (%)	Utilizable Carbohydrate (%)
LJRA	78.13 ± 1.49	6.70± 0.25	0.35± 0.06	5.05± 0.06	1.04± 0.17	8.73 ± 0.40
LJWA	74.39 ± 0.82	4.95± 0.15	1.76± 0.06	4.76± 0.08	1.46± 0.09	12.69 ± 0.36
WA (Parmar *et et*., 2017)	71.47	2.77	0.41	1.26	1.1	24.25
RA (Parmar *et al*., 2017)	78.76	3.58	0.26	0.95	1.12	16.27

LJRA = LibanJawi redanchote, LJWA= LibanJawi whiteanchote, RA=redanchote WA=whiteanchote

The mean percent protein content of red anchote sample was 6.70 mg/100g and the mean percent protein content of white anchote sample was 4.95mg/100g (Table 1). The protein content of red (6.70 mg/100 g) recorded was higher than the protein content (4.95 mg/100 g) of white anchote recorded. Generally; this showed red anchote have higher protein content than the white anchote. The mean percent moisture content of red anchote sample was 78.13 mg/100 g and the mean percent moisture content of white anchote sample was 74.47mg/100 g. The moisture content of red (78.13 mg/100 g) recorded was higher than the moisture content (74.47 mg/100 g) of whiteanchote recorded. Generally; this showed red anchote have higher moisture content than the white anchote. The mean percent of crude fiber content in redanchote (5.05 mg/100g) and the mean percent of crude fiber content in whiteanchote 4.76 mg/100 g sample were obtained respectively. The mean crude fiber content of redanchote (5.05 mg/100 g) obtained was higher than the crude fiber of whiteanchote (4.76 mg/100 g) obtained (Table 1). The mean percent of crude fat content in red anchote (0.35 mg/100 g) and the mean percent of crude fat content in whiteanchote1.76 mg/100g sample were recorded respectively. The mean crude fat content of red anchote (0.35 mg/100 g) documented was lower than the crude fat of white anchote (1.76 mg/100 g) recorded (Table 1).

The mean percent of total ash content documented in red anchote sample was 1.04 mg/100 g and mean percent of total ash content documented in white anchote sample was 1.46 mg/100 g (Table 1). The mean percent of total ash (1.0 4 mg/100g) of red anchote recorded was lower than the mean percent of total ash (1.46 mg/100g) of white. The mean percent of utilizable carbohydrate content obtained in red anchote sample was (8.73 mg/100 g) and mean percent of utilizable carbohydrate (12.69 mg/100g) were recorded respectively. The mean percent of utilizable carbohydrate content obtained in redanchote sample (8.73 mg/100 g) was lower than the percent content of utilizable carbohydrate (12.69 mg/100 g) in white anchote sample (Table 1). The nutritional and proximate composition value in white and red anchote were compared using one way ANOVA.

The percent of moisture content in red and white anchote tuber were 78.13 mg/g and 74.39 mg/100.The lower percent moisture content (74.39 mg/100g) was found in white anchote, whereas the higher moisture content was found (78.13mg/100g) in red anchote. Among the two samples, the highest moisture content (78.13mg/100g)

was recorded in red anchote, whereas the lowest moisture content (74.39 mg/100g) was recorded in white anchote (Table 1). However, one –way ANOVA test showed that there was significant difference ($P<0.05$) between the percent of moisture content in the anchote sample. The percent mean Crude protein content in red and whiteanchote samples were 6.70 mg/100 g and 4.95 mg/100 g (Table 1).The higher percent mean of crude protein (6.70mg/100g) was found in red anchote sample whereas the lower percent mean of protein (4.95mg/100g) was found in white anchote. Among the two samples, the highest Crude protein content (6.70 mg/100g) was red anchote, whereas the lowest Crude protein content (4.95 mg/100g) was white anchote (Table 1). However, one –way ANOVA test showed that there was significant difference ($P < 0.05$) between the percent of crude protein content in the anchote sample. The percent mean Crud e fat content in red and white anchote samples were 0.35 mg/100g and 1.76 mg/100g.The higher percent mean of crude fat (1.76 mg/100 g) was found in white anchote sample whereas the lower percent mean of fat (0.35mg/100 g) was found in red anchote. Among the two samples, the highest Crude fat content (1.76 mg/100 g) was white anchote; whereas the lowest crude fat content (0.35 mg/100g) was red anchote. However, one –way ANOVA test showed that there was no significant difference ($P>0.05$) between the percent of crude fat content in the anchote sample.

The percent mean of crude fiber content in white and redanchote samples were 4.76 mg/100g and 5.05 mg/100g (Table 1). The lower percent mean of crude fiber was found (4.76 mg/100g) in whiteanchote and the higher percent mean crude fiber content (5.05mg/100g) was found in redanchote. Among the two samples, the highest Crud fiber content (5.05 mg/100g) was redanchote, whereas the lowest crude fiber content (4.76mg/100g) was whiteanchote. However, one –way ANOVA test showed that there was no significant difference ($P>0.05$) between the percent of crude fiber content in the anchote sample. The percent mean total ash content in red and white anchote samples were 1.04 mg/100g and 1.46mg/100g.The higher percent mean of total ash (1.46 mg/100g) was found in whiteanchote sample whereas the lower percent mean total ash (1.04mg/100g) was found in redanchote.

Among the two samples, the highest total ash content (1.46 mg/100g) found in whiteanchote, whereas the lowest total ash

content (1.04 mg/100g) found in redanchote (Table 1). However, one –way ANOVA test showed that there was significant difference ($P<0.05$) between the percent of total ash content in theanchote sample. The percent mean of total utilizable carbohydrate content in white and redanchote samples were 12.69 mg/100g and 8.73mg/100g. The lower percent mean of total carbohydrate content was found (8.73 mg/100g) in redanchote and the higher percent mean total content (12.69mg/100g) was found in white. Among the two samples, the highest total carbohydrate content (12.69 mg/100g) was recorded in whiteanchote; whereas the lowest total content (8.73 mg/100g) was obtained in redanchote. However, one – way ANOVA test showed that there was significant difference ($P<0.05$) between the percent of total utiliziable carbohydrate content in theanchote sample.

Generally, out of the proximate of two varieties ofanchote sample, moisture content, crude protein, and crude fiber in redanchote were higher than that of whiteanchote. Redanchote of site of the study is an important source of diet like crude protein and crude fiber than whiteanchote. However, utilizable carbohydrate, crude fat and total ash in whiteanchote were higher than that of redanchote. Whiteanchote of site of the study are an important source of diet like crude fat and utilizable carbohydrate than redanchote. The difference in the amount of moisture content, crude protein, crude fiber, crude fat, total ash and total carbohydrate between the varieties ofanchote samples may be attributed due to the difference in source composition in the supportive soil, environment and agricultural practice.

Comparison of nutritional and proximate composition value of white and redanchote with similar studies

As indicated in Table 2, the moisture content of white and red anchote samples were 71.07 % and 78.76% for white and red respectively which is in agreement with the moisture content of white and redanchote reported in literatures (Parmar *et al*., 2017; Ayalew *et al*.,, 2016; Habtamu, 2013). On the other hand, the overall percent mean value of moisture content obtained from this work were greater than moisture content obtained by (Abera, 1995 ; Fekadu , 2014 ; EHNRI., 1997 and Habtamu, 1997) who reported values of, 74.4,74.93, 74.50, and 73.00 (%) respectively for anchote tuber. However, (Abera, 1995 ; Fekadu , 2014 ; EHNRI., 1997 and Habtamu, 1997) did not specify the tissue color of the anchote used for analysis.

The crude protein content of white and redanchote tuber in the present study was higher than the crude protein obtained by (Parmar *et al.*, 2017) for white, (2.77mg/100g) and redanchote (3.58 mg/100g). The crude protein content ofanchote tuber in the present study was to be moved between the range value (4.6-16.4 %) were reported by (Fekadu, 2011), but higher than the values (3.00-3.20 %) reported elsewhere (EHNRI, 1997; Habtamu, 2014 and Habtamu, 1997), however (Fekadu 2011; EHNRI. 1997 and Habtamu 2014), didn't specify the tissue color ofanchote, so comparison is difficult. The mean of crude fat obtained in this work, somewhat similar to the finding of (Fufa, 1997 ; Abera, 1995 and EHNRI, 1997), (0.17g/100 g), 0.12 g/100g, and 0.1 g/100 g), respectively. However; they did not specify the tissue color of theanchote used for analysis; hence, it is not possible to provide a further comparison with red and white accessions. On the other hand, the mean of crude fat obtained in this work, was higher than the finding of (Parmar *et al*., 2017).

The percent mean value of crude fiber content finding is in agreement with reported values (Ayalew *et a.l*, 2016) which is ranged from 3.63 ± 0.04 % to 6.96 ± 0.24 % and whereas higher than previously reported values foranchote tuber: namely 2.58 % (Fekadu e*t al.*, 2014), however, (Fekadu *et al.*, 2014), did not identify the tissue color of theanchote used for analysis; hence, it is not possible to provide further comparison of red and white accessions. Additionally in this study the Crude fiber content obtained was higher than that of (Parmar *et al.*, 2017), for white (1.26 mg/100g) and red (0.95mg/100g). The percent mean value of total ash contents finding in this study is lower than the findings of (Fekadu *et al.*, 2014) that were 2.19%, 2.00%, and 1.10%, respectively. However, (Fekadu *et al.*, 2014) did not specify the tissue color of theanchote used for analysis; hence, it is not possible to provide a comparison of red and white accessions. The percent mean value of total ash contents of red and whiteanchote more agreement with that of (Parmar *et al.*, 2017), which were done analyses on varieties of anchote. The percent mean value of total carbohydrate contents in this study was lower than that of finding by (Parmar *et al.*, 2017) which stated as 24 mg/100g in white and 16.27 mg/100g in redanchote. In similar way the value obtained from this study was lower than the finding of Fekadu *et al.*, 2014) which was 16.86 ±0.41 for raw, 10.42 ± 0.310 boil after peeling,1 ± 5.23 ± 0.40 boil before peeling ofanchote respectively ,however , Habtamu didn't

perform analysis of anchote based on tissue color they possess.

Digestion Procedure of white and red anchote

The digestion procedures and their corresponding observations are summarized in Table 3.

Some types of solid samples can be easily dissolved with a mixture of strong acids (for example, nitric and per chloric acids) and oxidants (commonly hydrogen peroxide). From the, digestion of 0.5 g of white and redanchote with mixtures of 5ml of HNO_3, 2 mL of $HClO_4$, and 1 mL H_2O_2 digested at a temperature of 240 ^{0}C for 3 hours gave a clear colorless solution. After the digestion the samples were cooled and diluted to 50 mL volumetric flask with deionized water. The instrument was calibrated using standard working series of solutions of each of the metals also prepared freshly by appropriate dilution of the intermediate standard solutions, which were prepared from stock solution of each metal and a concentration of the intermediate standards and working standard solutions of each metal. Concentration values of working standard solutions and correlation coefficients of calibration graph for White and Redanchote tuber was presented in Table 5.

Instrument working conditions and calibrations.

Instrument working condition

The Atomic absorption spectroscopy parameters for each element are shown in Table 4.

Instrument calibration

The concentrations of the calibration standards and value of the correlation coefficient of the calibration graph for each metal in which the FAAS was calibrated are listed in Table 5. Calibration curves for the different concentration range showed best linearity with coefficients of determination (r^2) ranging from 0.9985 to 0.9994(Table 5), which were obey acceptance limit of the regression line for the other (Christian, 2003). This shown that there is a fine relationship between concentration and an absorbance indicating good calibration of the instrument. This indicates that it is adequate to continue the analysis.

Method validation and quality controlling results

Instrument Detection Limit (IDL)

The Instrumental detection limits of all the metals of interest were computed from the responds of triplicate of the four calibration blanks using the respective regression equation of the calibration curve and equation (14). The values obtained ranged from 0.010 to 0.016 which were below the

method detection limit (Table 6) indicating, fine sensitivity of the measuring instrument for analysis.

Table 2. Digestion for 0.5g red and white anchote sample in Kjeldahel mothod.

anchote samples	Amount of sample (g)	Amount of reagent added (mL)	Digestion temp. (^oC)	Digestion Time (min)	Results
Red anchote	0.5	HNO_3 (70%), 5 mL $HCLO_4$ (70%), 2 mL H_2O_2 (35%), 1 mL	240	3	Clear solution
White anchote	0.5	HNO_3 (70%), 5 mL $HCLO_4$ (70%), 2 mL H_2O_2 (35%), 1 mL	240	3	Clear solution

Table 3. Working conditions of the atomic absorption spectroscopy.

Metal	wavelength (nm)	Slit width (nm)	Lamp current (mA)
Mg	285.2	0.5	5.0
Ca	422.7	0.5	5.0
K	767	0.5	2.0
Na	589	0.2	2.0

Table 4. Concentration of calibration standards, coefficient of determination (r^2) and calibration curves for the determination of metals in anchote

Metals	concentration of calibration Standard solution (ppm)	coefficient of determination (r^2)	calibration equation
Mg	1,2,3,4,5	0.9994	Y= 0.0636x - 0.0584
Ca	4,8,12,16,20	0.9994	Y = 0.0158x + 0.0062
K	0.8,1.4,2.4,3.2,4	0.9988	Y = 0.2816x - 0.0149
Na	1,2,3,4,5	0.9985	Y = 0.3138x - 0.0251

Method detection and quantification limits

The MDL and MQL for each metal analyte were computed from the instrument response of four blanks replicates of the

method blanks using the respective regression equation of the calibration curves and equation 15 and 16 respectively and the values are listed in Table 6. As observed from Table 6, the MDL values lied in the ranges between 0.011 and 0. 017. The MQL values lied in the between 0.063 and 0.142. The MDL estimated for the white and redanchote samples was low enough to detect the presence of metals of interest at trace levels inanchote tuber. The result verified that both MDL and MQL values were greater than the IDL; hence the results of analysis could be reliable.

Accuracy and precision

Accuracy of the method was determined by matrix spike recovery studies. The recovery values of the matrix spikeanchote samples were calculated as equation 13 and the results are presented in Table 7 and 8. As it can be seen in table 7 of the mean percent recovery values foranchote tuber ranged between 95 and 103 for all metals. The highest mean percent recovery (103) is for Ca and the lowest (95) is for Mg. All the recovery values ofanchote samples were within the designated acceptance range 80-120 % for metal analysis (Harvey, 2000). These results verify that the proposed method was accurate.

Table 5. IDL, MDL, and MQL for the determination of metals in anchote.

Metals	IDL (mg/Kg)	MDL (mg/Kg)	MQL (mg/Kg)
Na	0.013	0.014	0.063
K	0.016	0.017	0.095
Mg	0.010	0.011	0.142
Ca	0.013	0.014	0.065

Table 6. Recovery and precision test results of metals for anchote spike sample.

Metals	Conc.in sample mg/Kg	Amount added mg/Kg	Conc.in spiked sample mg/Kg	Recovery %	RSD
Na	5.70 ± 0.92	4	9.66 ± 0.56	99 ± 0.36	0.36
K	42.70±0.35	4	46.64 ±1.06	98.5 ± 0.19	0.19
Mg	0.29±0.20	4	4.09 ± 0.93	95±0.93	0.97
Ca	40.18±0.04	4	44.30 ± 0.89	103±0.93	0.90

Mean ±SD, n =3 RSD = relative standard deviation

Contamination control

Method blanks were run to identify and correct systematic errors due to impurities in the reagents and contamination in the glass ware and instrumentation. The analysis results verified that there was no reading above the IDL of the metals. Hence it can be concluded that the analytical method was free of overall laboratory contamination.

Laboratory control samples results

RSD and Laboratory control samples recoveries were calculated for each analyte using equation 12 and 17 respectively. The corresponding results are summarized in Table 8. As observed in Table 8, percent recovery values of LCS results lied in the range between 92.50 (in Na) and to 99(in Ca) fromanchote. And RSD ranged between 0.19 (in Ca) and 0.75 (Na in) fromanchote sample. All the values were found under recommended control limits 80-120% for laboratory control (LCS) recovery (Harvey, 2000) and ≤ 15 % for (RSD). These results showed the analytical method possess the required precision and accuracy (Csuros et al., 2016).

Table 7. Recovery and precision test results for the LCS of anchote at p=0.05 value.

Metals	Amount added mg/Kg	Conc.in spiked sample mg/Kg	Recovery %	RSD
Na	4	3.70±0.56	92.50 ±0.69	0.75
K	4	3.92±1.06	98. 00 ±0.41	0.42
Mg	4	3.73±0.15	93.25±0.32	0.34
Ca	4	3.96±0.89	99.00±0.19	0.19

Table 8. Mean concentrations of the studied essential metals in white and red anchote.

Metals	White anchote (mg/100 g)			Redanchote (mg/100 g)		
	Conc.	SD	RSD	Conc.	SD	RSD
Na	25.66	1.41	5.49	57.00	0.92	1.61
K	416.66	0.70	0.17	510	0.93	0.18
Ca	113.20	0.60	0.53	101.80	0.20	1.96
Mg	13.50	0.92	6.81	29.70	0.20	0.67

Determination of essential metals in white and redanchote

All the evaluated metal elements K, Ca, Na and Mg were determined with good precision, because they have shown with low precision (% RSD $\leq$ 15; - (Alemayehu *et al.*, 2013; Csuros *et al.*, 2016). Among the essential elements, the most abundant metal was K followed by Ca, Na and Mg, in red and whiteanchote respectively. Sodium is the major nutrient for human but non-essential alkali metal for plant was present in higher concentration next to calcium in all varieties ofanchote.

Levels of metals in white anchote and red anchote

Levels of metals in white and red anchote

The level of metal recorded in the present study in white and redanchote were Na (25.66 ± 1.41 mg/100 g, 57.00 ± 0.92 mg/100 g); K (416.66 ± 0.70 mg/100 g, 510 ± 0.93 mg/100 g); Ca(113.20 ± 0.60 mg/100 g, 101.80 ± 0.20 mg/100 g) and Mg(13.50 mg/100 g ± 0.92, 29.70 mg/100 g) respectively. Generally as observed from the Table 9, the concentration of abundant metal found in white and red anchote sample was arranged as increasing order of; Mg < Na < Ca < K.

This result shows anchote tuber could be a good sodium source. Potassium is an essential nutrient needed for maintenance of total body fluid volume, electrolyte balance and normal cell function. WHO recommends an increase in potassium intake from food to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults. WHO suggests a potassium intake of at least 90 mmol/day (3510 mg/day).The high potassium to sodium ratio contents may make it good potassium sodium balance in the human body and so that it protects against heart disease (WHO, 2004).

The current recommendation is to consume less than 2,400 milligrams (mg) of sodium a day. This is about 1 teaspoon of table salt per day. It includes all salt and sodium consumed, including sodium used in cooking (Thompson *et. al*, 1985).

Magnesium is included in the active transport across the cell membrane, which is an important process for conducting nerve impulses. The recommended daily intake of Mg for adults is about 400 mg. The high concentration of Ca is very significant because Ca is known to enhance the qualities of bones and teeth and also of neuromuscular systemic and cardiac functions. Thus including this tuber in the diet may contribute significant amount of Ca and Mg for the daily need. The required daily intake of Ca is set at 300 mg for women and 350 mg for men (Baah *et al.*,

2009). Therefore, consuming red and white anchote produced at studied area is safe.

Comparison levels of metals in white and red anchote

The sodium content in the white and red anchote samples were 25.66 mg/100 g and 57.00 mg/100 g respectively. The higher concentration of Sodium (57.00 mg/100 g) is found in Red anchote sample, whereas the lower concentration of sodium (25.66 mg/100 g) is found in white anchote sample. The mean concentration of the white and red anchote not more far from each other, so that, one way ANOVA test showed that there was significant difference ($P < 0.05$) among the mean concentration of Sodium in the white and red anchote samples. The potassium content in white and red anchote samples were 416.66 mg/100 g and 510.00 mg/100g respectively. The higher concentration of potassium (510.00 mg/100 g) is found in red anchote samples whereas the lower concentration of potassium (416.66mg/100 g) is found in white anchote samples, however one way ANOVA test showed that there was no significant difference ($P > 0.05$) among the mean concentration of Potassium in the white and red anchote samples. The magnesium content in white and redanchote samples were (13.5 mg/100g and 29.7 mg/100g) respectively. The higher concentration of magnesium (29.7mg/100g) is found in red anchote samples; whereas the lower concentration of magnesium (13.5 mg/100g) is found in white anchote samples, however One way ANOVA test showed that there was no significant difference ($P > 0.05$) among the mean concentration of magnesium in the white and red anchote samples.

The calcium content in red and white anchote samples were 101.8 mg/100g and 113.2 mg/100g respectively. The higher concentration of calcium (113.2 mg/100g) is found in white anchote samples; whereas the lower concentration of calcium (101.8 mg/100g) is found in red anchote samples. The mean concentration of the white and red anchote were close to each other, therefore, one way ANOVA test showed that there was significant difference ($P < 0.05$) among the mean concentration of Calcium in the white and red anchote samples.

Generally, out of the two variety of anchote samples, the highest concentrations of, K (510 mg/100 g) and K(416.66 mg/Kg), were determined in red and white anchote sample respectively. On the other hand, the lowest concentrations of Mg(29.7 mg/100 g) Mg(13.2 mg/100 g) were determined in red and white anchote sample respectively. The variation in the levels of metals between the

samples may be attributed to the difference in metal concentration in the supportive soil, environment and agricultural practice.

Table 9. Mean concentration of metals in 100 g (raw, peeled, unprepared, edible portion mean±SD, n = 3, dry weight) of white and red anchote with similar studies at P=0.05

Metals	White anchote mg/100 g	Red anchote mg/100 g	White[1] and red[2] anchote mg/100 g	Raw anchote[3] mg/100 g
Na	25.66±1.41	57.00 ±0.92	5.763, 5.87	42.78-98.78
K	416.66±0.7	510.00 ±0.3	315.83, 313.0	14.09-48.64
Ca	113.20±0.6	101.80 ±0.2	81.16, 59.13	80.64-372.16
Mg	13.50 ±0.92	29.70 ±0.20	50.30, 50.33	9.33-59.36

Source; [1, 2] Palmar *et al*., 2017, [3] Ayalew *et al.*, 2016

Comparison of the levels of metals in white and red anchote with similar studies

In this work, as can be seen from Table 10 the concentration of magnesium (13.50 mg/100 g) and (29.70 mg/100 g) obtained in both varieties was higher than the concentration of magnesium obtained by (Parmar, 2017) and much agreement with concentration of magnesium obtained by (Ayalew *et al*, 2016). However, Ayalew didn't perform analysis of anchote based on variety in tissues of color they possess, so comparison is much possible. On the other hand, the concentration of Potassium in the two variety of anchote 510.00 mg/100 g and 416.66 mg/100 g were higher than values reported by scholars (Parmar *et al.,* 2017; Ayalew *et al*., 2016). The concentrations of Calcium in both variety of anchote were higher than previously reported values (Parmar *et al*., 2017; Ayalew *et al.,* 2016). In contrast, the concentration of magnesium in both varieties of anchote was lower than the concentration of magnesium reported by other scholars (Parmar *et al.*, 2017; Ayalew *et al*., 2016).

Conclusion

In the present study the concentration levels of essential elements (Na, K, Mg and Ca) and the proximate composition values (moisture content, crude protein, crude fat, crude fiber, total ash and total carbohydrate), in white and red anchote tubers collected from LibanJawi Market of Babich District were determined by flame atomic absorption spectroscopy. The wet digestion procedures were developed for white and red anchote and analysis was performed. The efficient of procedures for

anchote (*Coccinia Abyssinica)* of the minerals, was evaluated through the recovery experiment, and a good percentage recovery was obtained for all of the mineral nutrients identified. Generally, out of the six nutritional and proximate compositions of two varieties of anchote sample, moisture content, crude protein, and crude fiber in red anchote were higher than that of white anchote. Red anchote of the study is an important source of diet like crude protein and crude fiber than white anchote. However, utilizable carbohydrate, crude fat and total ash in white anchote were higher than that of red anchote. White anchote of site of the study is an important source of diet like crude fat and utilizable carbohydrate than red anchote. On the other hand, the result of the present study indicated that both white and red anchote contain significant concentration of essential metals. Potassium was the most accumulated metal in the samples of two varieties ofanchote. Generally, out of the two variety of anchote samples, the highest concentrations of, K (510.00mg/100g, 416.66 mg/100g), were determined in red and white anchote sample respectively. The lowest concentrations of Mg (29.70mg/100g, 13.50mg/100g) were determined in red and white anchote sample respectively. The variation in the levels of metals between the samples may be attributed due to the difference in ages, in the supportive soil, environment and agricultural practice

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Conflict of interest

The authors declare that they have no conflict interest.

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