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Nutritional and phenolic profiles of leaves of fifteen Anchote (Coccinia abyssinica) accessions

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Abstract: The purpose of this study was to evaluate the nutritional and phenolic compositions of leaves of Anchote (Coccinia abyssinica) accessions grown in East Wollega Zone (Nekemte), Ethiopia. Leaves of fifteen accessions of Anchote were collected from research farm of Wollega University Ethiopia. The dried leaves of each accession were ground separately into fine powder following standard procedures. Thereafter, the proximate, mineral, and phenolic compositions were determined according to published official standard procedures with grade chemicals and reagents. Mineral ratios were considered to examine mineral-mineral interactions and antinutrient/mineral molar ratios were calculated to predict minerals bioavailability. Principal component analysis (PCA) and cluster analyses were conducted to characterize the accessions based on nutritional and mineral traits. Proximate compositions (% dwb) except moisture content were varied significantly (P < 0.05) and ranged: moisture 9.21–10.15, crude protein 16.88–29.84, crude fiber 8.52–12.07, crude fat 2.03–4.19, total ash 10.06–17.27, utilizable carbohydrate 37.45–46.36 and gross energy 272.67–293.11 kcal/100 g. PCA of proximate traits showed 74.15% variation for two principal components and the accessions categorized into three clusters. Mineral contents (mg/100 g dwb) were significantly varied (P < 0.05) and ranged: calcium (79.66–100.68), magnesium (31.18–68.47), sodium (41.32–71.95), potassium (71.79–111.13), phosphorus (42.07–66.9), Iron (3.68–11.51) and zinc (1.02–3.11). PCA of mineral traits showed 69.30% variation for two principal components and the accessions grouped into three clusters. Results of phenolic analyses were significantly varied

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PUBLIC INTEREST STATEMENT

Anchote leaf is one of the less known and neglected and native to Ethiopia particularly in Western part. In Ethiopia, it is growing in limited parts of the country and it is high productive when compared with the common edible leaves. Therefore, this neglected crop and traditional food for some specific part of the community in Ethiopia should be further studied and investigated for its nutritional and antinutritional compositions which will promote the crop for further cultivation and consumption.
(P < 0.05). Range of condensed tannin, phytate, total flavonoids and total phenols for the examined accessions were 129.0–255.32 mg/100 g, 218.46–240.67 mg/100 g, 8.03–12.74 mg/g QE, and 18.43–29.96 mg/g GAE, respectively. The study revealed that Anchote leaves contain appreciable amount of vital nutrients when compared to the commonly consumed green vegetables in Ethiopia. Particularly, those leaves of Anchote accessions that contained significantly higher amounts can be applied for breeding, new product development, and supplementation programs. Thus, the promotion of Anchote leaves for consumption and cultivation should be encouraged.

**Subjects:** Food Chemistry; Food Analysis; Nutrition

**Keywords:** Accession; anchote leaf; Coccinia abyssinica; proximate; phenolic

1. Introduction

The introductions of indigenous vegetables widely across rural and urban areas add variety and improve nutritional value of the diet (Fao, 2014). Most African countries, blessed with a variety of natural surroundings, varying climates and seasons, have a number of edible indigenous vegetables such as Anchote. Anchote (*Coccinia abyssinica*) is an endemic and potentially valuable crop of Ethiopia, primarily categorized under root and tuber crops (Ayalew et al., 2017; Holstein, 2012). It belongs to the order Cucurbitales, family Cucurbitaceae (Asfaw et al., 1992; Fekadu et al., 2013). There are about 10 species of Coccinia in Ethiopia; however, only *Coccinia abyssinica* is cultivated for human consumption (Bekele, 2007).

Anchote is cultivated mainly for its tuber though it has an edible leaves, which is used as nutritious vegetable served after being cooked (Abera, 1995; Ayalew et al., 2017). Anchote tuber is rich in protein, calcium, iron and phosphate contents with minimum antinutritional content, with the latter further reduced during cooking processes, the character that made it a nutritionally recommended food (Fufa & Urga, 1997; Tolera, 2017). However, it is such an underutilized vegetable that the compositions of different accessions of Anchote leaves were not thoroughly studied.

Hence, the populace is unaware of the value of Anchote leaves and rather it is regarded as traditional food mainly for its tubers and for low-income earners. Thus, they have not benefitted from the same level of research attention given to other vegetables and studying compositions of different accessions of Anchote leaves could provide awareness for promoting as an alternative means for dietary diversification. Therefore, the nutritional and phenolic compositions of leaves of Anchote accessions, which were identified, were studied.

2. Materials and methods

2.1. Experimental materials

Anchote leaves were collected from an identified fifteen accessions from Wollega University experimental field located in Nekemte, Ethiopia. The leaves samples were coded, packed in polyethylene bags, kept in an icebox and were transported to laboratory of Food Technology and Process Engineering, Wollega University. Analyses were conducted according to published official standard procedures with grade chemicals and reagents.

2.2. Sample preparation

Samples of leaves were cleaned with a dry cloth and moisture content was determined on a wet basis. The rest of the samples of leaves were rinsed with distilled water and were then air-dried at room temperature. The dried leaves were grounded separately into fine powder using electric grinder until to pass through 0.5 mm sieve mesh size. The powdered samples were stored in desiccator until analyses done after sealed into airtight polyethylene plastic bags.
2.2.1. Methods of analyses

2.2.1.1. Proximate compositions. The proximate analyses were conducted for contents of moisture, crude protein, crude fiber, crude fat, and total ash according to AOAC (2000). Official method 925.10 (air-oven drying method) used for moisture content determination while ES ISO 1871:2013 method [by Kjeldahl method through which Digestion (H₂SO₄), Distillation (NaOH) and Titration (HCl) processes involved] used for crude protein determination. The crude fiber content was determined according to official method 962.09 using acid/base digestion. Soxhlet extraction method (official method 920.39) used to extract and determine the crude fat content. Total ash content was determined according to official method 923.03 by incineration in a muffle furnace.

Utilizable carbohydrate content was calculated by difference basis (Gemede et al., 2016; Manzi et al., 2004). Atwater’s conversion factors [16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrate] used for calculating the energy value and expressed in calories (kcal per 100 g) (Gemede et al., 2016). The utilizable energy due to protein percentage [UEDP % = 60% * Percentage of Energy due to Protein (PEP %)] was also calculated to indicate the contribution of protein to the total energy (Ilesanmi & Jonathan, 2018).

2.2.1.2 Determination of mineral contents. The analyses of minerals were conducted according to AOAC (2000) by the principle of destroying organic matrix in a muffle furnace. Remaining ash was dissolved in diluted acid and the analyte concentration was determined according to Method Number (Modified AOAC 985.35) Atomic Absorption Spectrophotometer (PG Instruments Ltd, United Kingdom, and model PG-990) for calcium, zinc, and iron at an absorbance of 422.7 nm, 213.8 nm, and 248.3 nm, respectively. Method Number (Modified AOAC 966.16 and 965.30, respectively) used for sodium and potassium determination using flame photometry method. Phosphorus content was read at 660nm by using UV-visible spectrophotometer (DU-64 spectrophotometer, Beckman, USA) by complexing with molybdovanadate reagent according to Method (Modified AOAC 986.24).

2.2.2. Phenolic compositions determination

2.2.2.1. Phytic acid content determination. Latta and Eskin (1980) method as later modified by Vaintroub and Lapteva (1988) was used for determination of phytate after extraction of sample with 10 ml of 0.2 N HCl for 1 h, centrifuged, and reacting sample extract (3 mL) with 2 mL of Wade reagent (0.03% FeCl₃·6H₂O and 0.3% sulfosalicylic acid in distilled water). The absorbance of sample was measured at 500 nm using UV-Visible spectrophotometer (DU-64 spectrophotometer, Beckman). The amount of phytate content (mg/100 g sample) was calculated using phytic acid standard curve prepared in the same condition.

2.2.2.2. Condensed tannin content determination. Burns (1971) method as modified by Maxson and Rooney (1972) was used for determination of condensed tannin using vanillin-HCl reagent, and D-catechin as standard. One mL extracted sample (1 g of sample and 10 mL of 1% HCl in methanol, for 24 hr) reacted with 5 mL of vanillin-HCl reagent (8%concentrated HCl in methanol and 4% vanillin in methanol, 50:50, v/v), and the absorbance read at 450 nm using UV-visible spectrophotometer (DU-64 spectrophotometer, Beckman). Tannin concentration was calculated from the linear regression equation obtained and the result was expressed as condensed tannin content in mg/100 g dry weight.

2.2.2.3. Total flavonoids determination. Dowd method as adopted by Arvouet-Grand et al. (1994) was used to determine total flavonoids content colorimetrically using AlCl₃ and Quercetin as standard. One mL of 2% Aluminum trichloride (AlCl₃) in methanol mixed with the same volume of methanolic extracts (1000 µg) and after 10 min, the absorbance of the sample and blank (1 mL of extract solution with 1 mL methanol, without AlCl₃) measured. A quercetin calibration standard was prepared from 12.5 to 100 µg/ml of quercetin standard in methanol from 0.5 mg/ml stock.

2.2.2.4. Total phenol determination. Total phenols content was determined by Folin-Ciocalteu method as described by Singleton et al. (1999) using Gallic acid as standard. A total of 5 mL of
80% aqueous methanol was added, and the suspension stirred slightly. Tubes were sonicated for 40 min at 40°C in a sonicator bath and centrifuged (14,000 rpm for 10 minutes). Supernatants were collected and the amount of total phenolics in the extract was determined according to the Folin-Ciocalteu procedure. Absorption was read at 765 nm using UV-Visible spectrophotometer. Series of standard solutions were prepared using pure Gallic acid. Finally, the concentration was expressed as Gallic acid equivalents in mg/g of dry material.

2.2.3. Statistical analysis

Analyses were done in duplicates and the results obtained were statistically analyzed for one-way ANOVA using SPSS version 20.0 (SPSS Institute Inc., Cary, NC) for windows. Duncan multiple range tests were performed for analyzing significant differences (P < 0.05) among means as outlined by Obi (2002). Factor analysis based on principal component analysis (PCA) and cluster analyses were performed to characterize the accessions in relation to the most discriminating nutrient traits (Kubie, 2013).

3. Result and discussion

3.1. Proximate composition

The proximate analyses of 15 accessions of Anchote leaves studied are presented in Table 1. The table showed significant differences among the accessions (P < 0.05) in all proximate contents except in moisture content on a dry weight basis (% dwb) but significant difference (P < 0.05) obtained for moisture content on a wet weight basis (% ww). Accession “P18-Acc79” had the highest moisture content (82.16%) on a wet weight basis (% ww), which was significantly (P < 0.05) highest of all the studied accessions and accessions “P83-Acc186” (81.54%), “P109-Acc149” (81.44%), “P90-Acc162” (81.02%) and “P96-Acc69” (80.95%) followed on wet weight basis in that order. Whereas, accession “P93-Acc128” had significantly (P < 0.05) lowest (71.18%) moisture content of all the accessions on wet weight basis. The mean moisture content of the accessions was 79.65%, which was comparable to the leaves of Moringa oleifera 77.50% described by Vishwakarma and Dubey (2011) and Yam (Dioscorea spp.) 74.50% as reported by Gobezie et al. (1997) [26]. Leaves of Ipomoea batatas (84.33%) and Colocasia esculenta (90.00%) as studied by Vishwakarma and Dubey (2011) [25] showed a higher mean value. Akubugwo et al. (2007) reported that Amaranthus hybridus leaves (83.48 %) had a relatively higher mean value. Likewise, Gobezie et al. (1997) also reported higher moisture contents for the leaves of Cabbage (Brassica oleracea) 94.70%, Cauliflower (Brassica oleracea var. botrytis) 92.10%, Celery (Apium graveolens) 86.70%, Ethiopian kale (Brassicacarinata) 87.60%, Swiss chard (Beta vulgaris) 91.50%, Lettuce (Lactuca sativa) 95.50% and Spinach (Spinacea oleracea) 83.00%. “P97-Acc147” and “P10-Acc151” accessions had the highest and lowest moisture contents as 10.15 and 9.21%, respectively. Both were not significantly (P > 0.05) different from all the accessions. The mean moisture content of the accessions was 9.87%. A lower value of 8.56% reported for leaves of Anchote accessions by (Ayalew, 2016).Moisture content of any food is an index of its water activity (Frazier & Westoff, 1978; Nwoifa et al., 2012) and used as a measure of stability and the susceptibility to microbial contamination (Davey, 1989; Nwoifa et al., 2012).

The crude protein contents of the studied accessions are presented in Table 1. The crude protein content among the accessions varied significantly (P < 0.05) from 16.88% in “P4-Acc40” to 29.84% in “P85-Acc5” on a dwb. The accession “P85-Acc5” had significantly (P < 0.05) higher (29.84%) in crude protein content from all accessions except “P97-Acc147” with 29.47%. However, accession “P4-Acc40” had significantly (P < 0.05) lower (16.88%) crude protein content of all the accessions except “P90-Acc162” (17.48%), “P29-Acc178” (18.35%), “P3-Acc118” (19.07%), and “P109-Acc149” (19.14%). The mean crude protein content of the accessions was 21.60%. Lyaka et al. (2014) showed on his study on nutrient content of selected edible leafy vegetables that crude protein content is within the range of 27.71% & 34.20% for the edible green leafy vegetables. Ayalew (2016) also reported 20.50% crude protein content for leaves of Anchote accessions. Vishwakarma et al., (2011) reported lower crude protein content for leaves of Ipomoea batatas (13.50%), Moringa oleifera (9.94%) & Colocasia esculenta (3.23%). Gobezie et al. (1997) reported
Table 1. Proximate compositions mean value ± std. error (% on dwb)

| Accessions Name | Moisture | Crude Protein (%) | Crude Fiber (%) | Crude Fat (%) | Total Ash (%) | Utilizable Carbohydrate (%) | Gross Energy(kcal/100 g) |
|-----------------|----------|-------------------|----------------|--------------|---------------|-----------------------------|--------------------------|
|                 | % wwb    | % dwb             |                |              |               |                             |                          |
| P3-Acc118       | 80.06 ± 0.20<sup>a</sup> | 9.55 ± 0.4<sup>a</sup> | 19.07 ± 0.57<sup>cd</sup> | 9.8 ± 0.73<sup>cde</sup> | 3.58 ± 0.28<sup>abc</sup> | 16.91 ± 0.38<sup>bc</sup> | 41.10 ± 0.09<sup>cde</sup> | 272.86 ± 4.6<sup>f</sup> |
| P10-Acc151      | 79.29 ± 0.14<sup>ef</sup> | 9.21 ± 0.41<sup>a</sup> | 21.80 ± 0.62<sup>cd</sup> | 8.7 ± 0.62<sup>a</sup> | 2.89 ± 0.71<sup>abcd</sup> | 17.27 ± 0.24<sup>bc</sup> | 40.14 ± 1.78<sup>def</sup> | 273.73 ± 1.75<sup>f</sup> |
| P18-Acc79       | 82.16 ± 0.46<sup>a</sup> | 10.01 ± 0.36<sup>a</sup> | 22.06 ± 0.67<sup>cd</sup> | 11.91 ± 0.38<sup>a</sup> | 3.02 ± 0.37<sup>abcd</sup> | 12.55 ± 0.79<sup>cdde</sup> | 40.46 ± 0.54<sup>abcd</sup> | 277.22 ± 2.05<sup>cde</sup> |
| P25-Acc140      | 79.47 ± 0.26<sup>ef</sup> | 9.47 ± 0.27<sup>a</sup> | 20.11 ± 0.82<sup>ab</sup> | 10.77 ± 0.54<sup>abc</sup> | 2.53 ± 0.48<sup>abcd</sup> | 10.47 ± 0.34<sup>ef</sup> | 43.99 ± 0.28<sup>abcd</sup> | 279.13 ± 3.56<sup>cde</sup> |
| P55-Acc111      | 79.51 ± 0.21<sup>ef</sup> | 10.04 ± 0.41<sup>a</sup> | 26.15 ± 0.58<sup>a</sup> | 10.36 ± 0.15<sup>cde</sup> | 2.03 ± 0.26<sup>a</sup> | 13.97 ± 0.81<sup>bc</sup> | 37.45 ± 0.87<sup>f</sup> | 272.67 ± 3.5<sup>f</sup> |
| P80-Acc201      | 78.68 ± 0.29<sup>abc</sup> | 9.83 ± 0.12<sup>a</sup> | 23.16 ± 0.86<sup>c</sup> | 9.12 ± 0.83<sup>cd</sup> | 3.22 ± 0.17<sup>abcd</sup> | 13.89 ± 0.87<sup>bc</sup> | 40.78 ± 0.51<sup>cdef</sup> | 284.74 ± 0.13<sup>cde</sup> |
| P90-Acc162      | 81.02 ± 0.19<sup>bc</sup> | 9.95 ± 0.20<sup>a</sup> | 17.48 ± 0.92<sup>f</sup> | 8.52 ± 0.22<sup>a</sup> | 3.18 ± 0.33<sup>abcd</sup> | 14.51 ± 0.91<sup>b</sup> | 46.36 ± 0.10<sup>cde</sup> | 283.98 ± 0.32<sup>cde</sup> |
| P96-Acc69       | 80.95 ± 0.18<sup>bc</sup> | 10.10 ± 0.33<sup>a</sup> | 20.22 ± 0.86<sup>ab</sup> | 12.07 ± 0.19<sup>a</sup> | 3.94 ± 0.84<sup>a</sup> | 11.48 ± 0.59<sup>def</sup> | 39.35 ± 0.25<sup>cde</sup> | 273.7 ± 5.16<sup>cde</sup> |
| P97-Acc147      | 78.39 ± 0.28<sup>h</sup> | 10.15 ± 0.21<sup>a</sup> | 29.47 ± 0.54<sup>de</sup> | 9.16 ± 0.08<sup>de</sup> | 2.07 ± 0.11<sup>a</sup> | 14.33 ± 0.38<sup>b</sup> | 37.68 ± 1.37<sup>f</sup> | 287.19 ± 2.33<sup>cde</sup> |
| P109-Acc149     | 81.44 ± 0.28<sup>bc</sup> | 10.03 ± 0.17<sup>a</sup> | 19.14 ± 0.61<sup>c</sup> | 11.61 ± 0.43<sup>abc</sup> | 3.95 ± 0.22<sup>a</sup> | 13.58 ± 0.94<sup>bc</sup> | 41.70 ± 1.59<sup>cde</sup> | 278.87 ± 5.9<sup>bcde</sup> |
| P14-Acc40       | 79.77 ± 0.22<sup>ef</sup> | 9.92 ± 0.11<sup>a</sup> | 16.88 ± 0.51<sup>f</sup> | 10.95 ± 0.34<sup>abc</sup> | 3.78 ± 0.17<sup>bc</sup> | 13.82 ± 0.61<sup>bc</sup> | 44.65 ± 0.72<sup>de</sup> | 280.14 ± 2.3<sup>bcde</sup> |
| P83-Acc186      | 81.54 ± 0.18<sup>bc</sup> | 9.88 ± 0.14<sup>a</sup> | 19.88 ± 0.83<sup>ab</sup> | 12.02 ± 0.38<sup>bc</sup> | 4.19 ± 0.14<sup>a</sup> | 10.06 ± 0.08<sup>f</sup> | 43.87 ± 1.57<sup>cde</sup> | 293.11 ± 1.7<sup>e</sup> |
| P29-Acc178      | 80.81 ± 0.26<sup>cd</sup> | 10.02 ± 0.34<sup>a</sup> | 18.35 ± 0.62<sup>f</sup> | 10.98 ± 0.63<sup>abc</sup> | 3.09 ± 0.35<sup>abcd</sup> | 12.03 ± 0.54<sup>ef</sup> | 45.53 ± 0.54<sup>cde</sup> | 283.33 ± 3.5<sup>cde</sup> |
| P93-Acc128      | 71.18 ± 0.39<sup>ef</sup> | 10.12 ± 0.13<sup>a</sup> | 20.31 ± 0.83<sup>ef</sup> | 11.07 ± 0.10<sup>abc</sup> | 4.07 ± 0.44<sup>a</sup> | 13.94 ± 0.75<sup>bc</sup> | 40.50 ± 0.75<sup>def</sup> | 279.81 ± 4.26<sup>cde</sup> |
| P85-Acc5        | 80.57 ± 0.36<sup>cde</sup> | 9.83 ± 0.14<sup>a</sup> | 29.84 ± 0.61<sup>de</sup> | 9.84 ± 0.08<sup>de</sup> | 2.28 ± 0.12<sup>cd</sup> | 13.16 ± 0.68<sup>bcd</sup> | 37.75 ± 0.36<sup>def</sup> | 290.86 ± 0.12<sup>cd</sup> |
| Mean            | 79.65    | 9.87              | 21.60          | 10.46        | 3.19           | 13.46                      | 41.42                     | 280.76                    |

Note: Number of sample size is fifteen and replicates are three.
a lower protein contents from the present study for commonly consumed vegetables in Ethiopia: as 0.90 g/100 g, 2.70 g/100 g, 3.30 g/100 g, 2.8 g/100 g, 2.2 g/100 g, 1.0 g/100 g, 5.6 g/100 g and 3.1 g/100 g. The reported values are for the leaves of Cabbage (Brassica oleracea), Cauliflower (Brassica oleracea var. botrytis), Celery (Apium graveolens), Ethiopian kale (Brassica carinata), Swiss chard (Beta vulgaris), Lettuce (Lactuca sativa), Spinach (Spinacea oleracea) and Yam (Dioscorea spp.), respectively. However, Getachew et al. (2013) reported a higher value for leaves of Coccinia grandis (36.3%).

The crude fiber contents of the studied accessions are presented in Table 1. The crude fiber content among the accessions varied from 8.52% to 12.07% in “P90-Acc162” and “P96-Acc69” accessions, respectively. The accession “P96-Acc69” had significantly (P < 0.05) higher (12.07%) crude fiber content than all accessions except “P83-Acc186” (12.02%), “P18-Acc79” (11.91%), “P109-Acc149” (11.61%), “P93-Acc128” (11.07%), “P29-Acc178” (10.98%), “P4-Acc40” (10.95%) and “P25-Acc140” (10.77%). However, the accession “P90-Acc162” had significantly (P < 0.05) lower (8.52%) crude fiber content than all the accessions except “P10-Acc151” (8.70%), “P80-Acc201” (9.12%), “P97-Acc147” (9.16%), “P3-Acc118” (9.8%) and “P85-Acc5” (9.84%) on dry weight basis. The mean fiber content in the accessions was 10.46%, which was higher when compared to content of other leafy vegetables such as Ipomoea batatas (1.42%) and Moringa oleifera (0.9%) as reported by Vishwakarma and Dubey (2011). However, Vishwakarma and Dubey (2011) also reported a comparable result for Colocasia esculenta (9.43%) and Getachew et al. (2013) for Coccinia grandis (10.1%). Ayalew (2016) also reported 11.42% mean crude fiber content for leaves of Anchote accessions. A study by Gobezie et al. (1997) showed that the commonly consumed vegetables in Ethiopia had fiber compositions as 0.90 g/100 g, 1.30 g/100 g, 1.90 g/100 g, 1.50 g/100 g, 1.1 g/100 g, 0.7 g/100 g, 1.6 g/100 g, and 1.7 g/100 g. The reported values are for leaves of Cabbage (Brassica oleracea), Cauliflower (Brassica oleracea var. botrytis), Celery (Apium graveolens), Ethiopian kale (Brassica carinata), Swiss chard (Beta vulgaris), Lettuce (Lactuca sativa), Spinach (Spinacea oleracea) and Yam (Dioscorea spp.), respectively. Those values were lower than the leaves of Anchote accessions under study. The fiber content in the leaves can still make significant contribution to dietary intakes since fiber contributes to lowering of plasma cholesterol levels, decreasing the incidence of colon cancer, lowering insulin requirements of diabetics, and in softening stool (Gemede et al., 2016; Ikewuchi & Ikewuchi, 2008). It also plays a physiological role in maintaining the internal distension for a normal peristaltic movement of intestinal tract, to ensure the smooth movement of food through the digestive tract (Hanif et al., 2006; Lyaka et al., 2014).

Crude fat contents of all the accessions under study are listed in Table 1. Accession “P83-Acc186” had the highest crude fat content (4.19%) which was significantly (P < 0.05) higher than the crude fat content of the accessions “P25-Acc140” (2.53%), “P85-Acc5” (2.28%), “P97-Acc147” (2.07%) and “P55-Acc111” (2.03%) on dry weight basis in that order. Whereas, accession “P55-Acc111” had significantly (P < 0.05) lower (2.03%) crude fat content of the accessions “P3-Acc118” (3.58%), “P4-Acc40” (3.78%), “P96-Acc69” (3.94%), “P109-Acc149” (3.95%) and “P93-Acc128” (4.07%) on dry weight basis. The mean crude fat content of the accessions was 3.19%. Getachew et al. (2013) reported a comparable value for leaves of Coccinia grandis (3.5%) whereas a higher value for Amaranthus hybridus leaves (4.65%) reported by Akubugo et al. (2007). Ayalew (2016) also reported 3.63% mean crude fat content for leaves of Anchote accessions. A much lower value than the present study for the leaves of commonly consumed vegetables such as Cabbage (Brassica oleracea) 0.1 g/100 g, Celery (Apium graveolens) 0.5 g/100 g, Ethiopian kale (Brassicaracinita) 0.8 g/100 g, Spinach (Spinacea oleracea) 0.8 g/100 g was reported (Gobezie et al., 1997). Lintas (1992) stated on his book on nutritional aspects of fruits and vegetables consumptions that the concentration of lipid ranged from 9.32% to 11.91%, which is in agreement with the general observation that leafy vegetables are low lipid-containing foods that plays significant role in avoiding obesity (Lintas, 1992; Lyaka et al., 2014). Excess fat consumption has been implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging (Antial et al., 2006).
The total ash contents of all the accessions are presented in Table 1. The level of ash content ranged from 10.06% (P83-Acc186) to 17.27% (P10-Acc151). The ash content was significantly (P < 0.05) higher in “P10-Acc151” (17.27%) than all the accessions except “P3-Acc118” (16.91%). However, accession “P83-Acc186” had significantly (P < 0.05) lower (10.06%) ash content than all the accessions except “P25-Acc140” (10.47%), “P96-Acc69” (11.48%) and “P29-Acc178” (12.03%) on dry weight basis. The mean ash content for the accessions was 13.46%. Akubugo et al. (2007) stated comparable ash content 13.80% for Amaranthus hybridus accessions. Coccinia grandis leaves (15.2 %) had a slightly higher mean value as reported by Getachew et al. (2013). Ayalew (2016) also reported 11.96% mean value total ash content for leaves of Anchothe accessions. On the other hand, Gobezie et al. (1997) reported the total ash content for the leaves of commonly consumed vegetables such as Cabbage (Brassica oleracea), Cauliflower (Brassica oleracea var. botrytis), Celery (Apium graveolens), Ethiopian kale (Brassica oleracea var. var. botrytis), Swiss chard (Beta vulgaris), Lettuce (Lactuca sativa), Spinach (Spinacea oleracea) and Yam (Dioscorea spp.). The values were 0.30, 0.90, 2.0, 1.9, 2.1, 0.5, 4.2, and 1.1%, respectively, that was lower than the present study for Anchothe leaves. The proportion of ash is a reflection of the mineral contents of the food material (Nwofia et al., 2012; Omotosho, 2005). Therefore, the total ash content is directly proportional with inorganic element content of Anchothe leaves. Hence, the samples with high percentage ash contents expected to have high concentrations of various mineral elements, which are advantage to speed up metabolic processes and improve growth and development (Fufa & Urga, 1997).

The utilizable carbohydrate contents of the studied accessions are presented in Table 4.1. The utilizable carbohydrate contents of accessions varied from 37.45% to 46.36% in “P55-Acc111” and “P90-Acc162” accessions, respectively. Utilizable carbohydrate content of accession “P90-Acc162” had significantly (P < 0.05) higher (46.36%) than all accessions except “P29-Acc178” (45.53%), “P4-Acc40” (44.65%), “P25-Acc140” (43.99%) and “P83-Acc186” (43.87%). Besides, accession “P55-Acc111” had significantly (P < 0.05) lower (37.45%) utilizable carbohydrate content than accessions “P83-Acc186” (43.87%), “P25-Acc140” (43.99%), “P4-Acc40” (44.65%) and “P29-Acc178” (45.53%) on dry weight basis. The mean value of the accessions was 41.42%, which was higher than the values of leaves of Colocasia esculenta (18.72%) and Moringa oleifera (18.72%) as reported by Vishwakarma and Dubey (2011). Amaranthus hybridus accessions (52.18%) showed a higher carbohydrate content than this study as stated by Akubugo et al. (2007). Getachew et al. (2013) reported a comparable 34.9% for leaves of Coccinia grandis. Ayalew (2016) also reported 52.56% mean value of utilizable carbohydrate content for leaves of Anchothe accessions.

The gross energy values of the studied accessions are presented in Table 1. Gross energy values were ranged from 272.67 kcal/100 g in “P55-Acc111” to 293.11 kcal/100 g in “P83-Acc186” accessions. Gross energy content of accession “P83-Acc186” had significantly (P < 0.05) higher (293.11 kcal/100 g) than all accessions except “P85-Acc5” (290.86 kcal/100 g), “P97-Acc147” (287.19 kcal/100 g), “P80-Acc201” (284.74 kcal/100 g), “P90-Acc162” (283.98 kcal/100 g) and “P29-Acc178” (283.33 kcal/100 g). However, accession “P55-Acc111” had lower (272.67 kcal/100 g) gross energy content which did not differ significantly (P < 0.05) from all the accessions except “P85-Acc5” (290.86 kcal/100 g), “P97-Acc147” (287.19 kcal/100 g), “P80-Acc201” (284.74 kcal/100 g) and “P90-Acc162” (283.98 kcal/100 g) on dry weight basis. The mean value of the study was 280.76 kcal/100 g, which was than Diplazium esculentum (195.00 kcal/100 g) but lower when compared to leaves of Amaranthus viridis (541.33 kcal/100 g), Chenopodium album (425.33 kcal/100 g), Basella rubra (375.00 kcal/100 g) and Basella alba (402.66 kcal/100 g) as reported by Saha et al. (2015). Ayalew (2016) also reported 325.05 kcal/100 g mean value of gross energy content for leaves of Anchothe accessions. A higher values for the leaves of Telfairia occidentalis (354.20 kcal/100 g), Moringa oleifera (363.60 kcal/100 g) and Brassica oleracea (319.80 Kcal/100 g) and a comparable value were informed for water Spinach leaves (300.94 kcal/100 g) by Umar et al. (2007). The value of utilizable energy due to protein (UEDP) % varied from 14.46% for accession P4-Acc40 to 24.62% for accessions P85-Acc5 and P97-Acc147. Therefore, leaves of Anchothe can be good source of protein with the evidence that confirms any plant foods, which
have the potential to provide about 12.00% of their calorific value from protein, are considered good source of protein (Aylew et al., 2017; Nwofia et al., 2012).

5.2. Principal component analysis of proximate nutrient traits

The results of the principal component analysis (PCA) of the six proximate values measured (Table 2) showed that the first two components contributed 74.15% of variability among the 15 accessions evaluated. PC 2 comprises three accessions (P97-Acc147, P85-Acc5, and P55-Acc111) while PC 1 consists of the others with 31.79% & 42.36% of variation, respectively.

The first principal component had high positive loading for crude fat, utilizable carbohydrate & total ash. The second principal component weighed the highest and positive moisture, crude fiber, and influenced by crude protein content. In this study, principal component analysis (PCA) was performed to define the existing pattern of variation among the accessions. Prasad et al. (2010) reported that PCA helps to identify traits that have substantive and meaningful contribution towards the observed variations. The scatter plots of the accessions on the principal component were presented in Figure 1.

Figure 1. Clustering based on principal components for proximate nutrient traits

As shown in Figure 1, cluster1 contained accessions (P3-Acc118, P10-Acc151, P80-Acc201, and P90-Acc162) with higher loading for total ash and lowest in crude fiber (P10-Acc151 and P90-Acc162). Cluster 2 consisted of accessions (P96-Acc69, P83-Acc186, P18-Acc79, P109-Acc149, P93-Acc128, P29-Acc178, P4-Acc40, and P25-Acc140) with higher crude fiber and crude fat (except P25-Acc140) contents whereas total ash (in P83-Acc186, P25-Acc140, P96-Acc69 & P29-Acc178) and crude protein (except in P18-Acc79) contents were the lowest value. Cluster 3 had accessions (P97-Acc147, P85-Acc5, and P55-Acc111) with high loading for crude protein content while lowest values for crude fat were observed. It was also reported that cluster analysis could be done for combining observations into homogenous group with respect to certain characteristics (Bozokalfa et al., 2011).

The mean values of accessions in each cluster are presented in Table 3. The first cluster comprises four accessions (P3-Acc118, P10-Acc151, P80-Acc201, and P90-Acc162) and showed highest mean value for total ash (15.65%) whilst lowest value for crude fiber (9.04%) and moisture content (9.64%). The second cluster consisted of eight accessions (P96-Acc69, P83-Acc186, P18-Acc79, P109-Acc149, P93-Acc128, P29-Acc178, P4-Acc40, and P25-Acc140), which had the highest mean value in crude fiber (11.42%), crude fat (3.57%) and utilizable carbohydrate (42.51%). Total ash (12.24%) and crude protein (19.63%) contents were the lowest value. The highest value for crude protein (28.49%) and moisture (10.01%) contents were obtained from the third cluster, which comprises three accessions (P97-Acc147, P85-Acc5, and P55-Acc111), while lowest values for crude fat (2.13%) and utilizable carbohydrate (37.63%) were also observed.

3.2. Mineral compositions

All mean results of mineral compositions of the accessions, which were selected based on the total ash content as an indicator for better mineral composition and studied, are compiled in Table 4. The concentrations of Calcium in the samples varied from 79.66 mg/100 g to 100.68 mg/100 g in “P90-Acc162” and “P4-Acc40” accessions, respectively. Accession “P4-Acc40” had the highest Calcium content (100.68 mg/100 g) which was significantly (P < 0.05) higher than all the accessions except accession “P55-Acc111” (100.63 mg/100 g). Accession “P90-Acc162” had significantly (P < 0.05) lower (79.66 mg/100 g) Calcium content than all the accessions except “P80-Acc201” (79.86%) on dry weight basis. The mean value of calcium content was 89.36 mg/100 g, which was comparable to leaves of Swiss chard (Beta vulgaris) 85.0 mg/100 g as reported by Gobege et al. (1997). However, the mean value was higher than the leaves of commonly consumed vegetables in Ethiopia: Cabbage (Brassica oleracea) 43.0 mg/100 g, Cauliflower (Brassica oleracea var. botrytis) 30.0 mg/100 g and Lettuce (Lactuca sativa) 22.0 mg/100 g as reported by
On the other hand, Gobezie et al. (1997) showed some of the commonly consumed vegetables leaves such as Celery (Apium graveolens) 317.0 mg/100 g, Ethiopian kale (Brassica carinata) 260.0 mg/100 g, Spinach (Spinacea oleracea) 122.0 mg/100 g and Yam (Dioscorea spp.) 119.0 mg/100 g had a higher mean value than the accessions of Anchote leaves under study. Ayalew (2016) reported the mean value of calcium content 147.8 mg/100 g for the leaves of Anchote accessions. Calcium is an important mineral for human beings, which provides good strength of bones and teeth (Amagloh & Nayarko, 2012; Saha et al., 2015). It plays an important role in blood clotting, muscles contraction, and neurological function and helps in
| Name of Accessions | Ca (mg/100 gm) | Mg (mg/100 gm) | Na (mg/100 gm) | K (mg/100 gm) | P(mg/100 gm) | Fe (mg/100 gm) | Zn (mg/100 gm) |
|-------------------|----------------|----------------|----------------|--------------|--------------|---------------|---------------|
| P3-Acc118         | 91.48 ± 0.23<sup>a</sup> | 54.02 ± 0.47<sup>d</sup> | 50.91 ± 0.13<sup>c</sup> | 73.44 ± 1.14<sup>f</sup> | 51.48 ± 1.03<sup>b</sup> | 4.88 ± 0.25<sup>g</sup> | 2.60 ± 0.13<sup>e</sup> |
| P10-Acc151        | 97.58 ± 0.27<sup>b</sup> | 58.59 ± 0.29<sup>c</sup> | 53.21 ± 0.96<sup>d</sup> | 92.38 ± 0.96<sup>d</sup> | 54.18 ± 0.10<sup>c</sup> | 5.21 ± 0.47<sup>g</sup> | 3.11 ± 0.68<sup>a</sup> |
| P85-Acc5          | 93.78 ± 0.17<sup>c</sup> | 68.47 ± 1.36<sup>c</sup> | 58.40 ± 0.37<sup>c</sup> | 102.00 ± 0.37<sup>c</sup> | 66.90 ± 0.13<sup>g</sup> | 8.07 ± 0.11<sup>cd</sup> | 1.98 ± 0.08<sup>de</sup> |
| P55-Acc111        | 100.63 ± 0.28<sup>b</sup> | 38.56 ± 0.68<sup>f</sup> | 57.12 ± 0.36<sup>c</sup> | 108.05 ± 0.95<sup>d</sup> | 46.89 ± 0.58<sup>g</sup> | 10.15 ± 0.62<sup>de</sup> | 1.94 ± 0.17<sup>bcde</sup> |
| P80-Acc201        | 79.86 ± 0.34<sup>h</sup> | 33.49 ± 0.29<sup>b</sup> | 52.60 ± 0.53<sup>e</sup> | 91.75 ± 1.43<sup>d</sup> | 62.94 ± 0.92<sup>b</sup> | 6.38 ± 1.01<sup>def</sup> | 2.80 ± 0.41<sup>de</sup> |
| P90-Acc162        | 79.66 ± 0.46<sup>n</sup> | 41.40 ± 0.04<sup>b</sup> | 48.03 ± 0.65<sup>f</sup> | 85.45 ± 0.84<sup>e</sup> | 49.30 ± 0.08<sup>f</sup> | 7.13 ± 0.82<sup>de</sup> | 3.04 ± 0.11<sup>de</sup> |
| P97-Acc147        | 86.04 ± 0.33<sup>c</sup> | 31.18 ± 0.14<sup>n</sup> | 68.01 ± 0.58<sup>e</sup> | 109.22 ± 0.44<sup>de</sup> | 46.65 ± 0.08<sup>g</sup> | 11.51 ± 0.12<sup>ef</sup> | 1.02 ± 0.14<sup>de</sup> |
| P109-Acc149       | 81.29 ± 0.37<sup>n</sup> | 53.84 ± 0.11<sup>e</sup> | 71.95 ± 0.11<sup>o</sup> | 111.13 ± 0.66<sup>n</sup> | 60.79 ± 0.33<sup>cd</sup> | 9.15 ± 0.53<sup>de</sup> | 2.27 ± 0.05<sup>de</sup> |
| P4-Acc149         | 100.68 ± 0.38<sup>c</sup> | 53.84 ± 0.40<sup>n</sup> | 48.77 ± 0.92<sup>f</sup> | 106.73 ± 0.88<sup>n</sup> | 50.99 ± 0.09<sup>n</sup> | 3.68 ± 0.16<sup>g</sup> | 1.11 ± 0.04<sup>cd</sup> |
| P93-Acc128        | 82.57 ± 0.29<sup>g</sup> | 31.96 ± 0.18<sup>n</sup> | 41.32 ± 0.25<sup>g</sup> | 71.79 ± 0.30<sup>f</sup> | 42.07 ± 1.06<sup>n</sup> | 5.70 ± 0.33<sup>ef</sup> | 2.24 ± 0.27<sup>de</sup> |
| **Mean**          | **89.36** | **47.24** | **55.03** | **95.29** | **53.22** | **7.19** | **2.21** |

Note: Number of sample size is fifteen and replicates are three.
enzymatic metabolic processes. Calcium plays an important role in building strong as well as in the keeping healthy bones and teeth at both early and later life (Senga Kitumbe et al., 2013).

Magnesium contents in the accessions are listed in Table 4. The contents of Magnesium varied from 31.18 mg/100 g in “P109-Acc149” to 68.47 mg/100 g in “P55-Acc111”. Accession “P55-Acc111” had the highest Magnesium content (68.47 mg/100 g) which was significantly (P < 0.05) higher than all the accessions and was followed by “P85-Acc5” (60.95 mg/100 g), “P10-Acc151” (58.59 mg/100 g), “P3-Acc118” (54.02 mg/100 g) and “P4-Acc640” (53.84 mg/100 g) in that order. However, the accession “P109-Acc149” had significantly (P < 0.05) lowest (31.18 mg/100 g) Magnesium content on dry weight basis. The mean value of magnesium content in this study was 47.24 mg/100 g, which was higher than the leaves of Amaranthus hybridus (34.12 mg/100 g) reported by Nyonje (2015) but comparable with Mucuna poggie leaves content (56.05 mg/100 g). Ayalew (2016) reported the mean value of magnesium content 45.04 mg/100 g for the leaves of Anchoate accessions. Magnesium, which is an essential to the function of several enzyme systems, is important in maintaining electrical potential in nerves and membranes, is involved with liberation of energy for muscle contraction, and is required for normal metabolism of calcium and phosphorus. It is also an important mineral element in connection with its role in circulatory diseases such as ischemic heart disease and calcium metabolism in bone (Hassan & Umar, 2006; Ishida et al., 2000).

Sodium contents of the accessions are presented in Table 4. In this study, the Sodium content varied from 41.32 mg/100 g “P93-Acc128” to 71.95 mg/100 g “P109-Acc149”. Sodium content of accession “P109-Acc149” was significantly (P < 0.05) highest (71.95 mg/100 g) while accession “P93-Acc128” had the lowest (41.32 mg/100 g) Sodium content which was significantly (P < 0.05) different from all accessions on dry weight basis. The mean value of Sodium in the study was 55.03 mg/100 g. Ayalew (2016) also reported the mean value of sodium content 54.46 mg/100 g for the leaves of Anchoate accessions. Sodium is an important mineral, which assists in the regulation of the body fluids and maintenance of electrolyte balance in the body. It is the chief extracellular ions of the body but excessive intake of can contribute to elevating blood pressure (Saha et al., 2015).

Potassium contents of the accessions are listed in Table 4. The value of potassium was with the range of 71.79 mg/100 g for P93-Acc128 accession to 111.13 mg/100 g for accession P109-Acc149. The Potassium content of accession “P109-Acc149” had significantly (P < 0.05) higher (111.13 mg/100 g) than all accessions except “P97-Acc147” (109.22 mg/100 g), whereas accession “P93-Acc128” had significantly (P < 0.05) lower (71.79 mg/100 g) content than all accessions except “P3-Acc118” (73.44 mg/100 g) on dry weight basis. This study showed a mean value of 95.29 mg/100 g. Saha et al. (2015) reported on his study on leaves of eight green leafy vegetables that Moringa oleifera contained 75.33 mg/100 g and Chenopodium album 70.70 mg/100 g, the rest had the potassium contents in the range of 71.36–74.46 mg/100 g, which was comparable to the present study. Ayalew (2016) also reported the mean value of potassium content 139.82 mg/100 g for the leaves of Anchoate accessions. Potassium, which is the principal intracellular cation, is involved with cellular enzyme function and with Sodium helps to regulate osmotic pressure and pH equilibria. It is also essential for life and high amount of potassium increases the Iron utilization and is beneficial to the people taking diuretics to control hypertension as reported by Archana et al. (2012).

Phosphorus contents of the accessions are presented in Table 4. In this study, the phosphorus content ranged from 42.07 mg/100 g “P93-Acc128” to 66.90 mg/100 g “P85-Acc5”. Accession “P85-Acc5” had the highest Phosphorus content (66.90 mg/100 g), which was significantly (P < 0.05) highest and was followed by “P80-Acc201” (62.94 mg/100 g), “P109-Acc149” (60.79 mg/100 g) and “P10-Acc151” (54.18 mg/100 g) in that order. However, the accession “P93-Acc128” had significantly (P < 0.05) lowest (42.07 mg/100 g) Phosphorus content on dry weight basis. The mean value of the study was 53.22 mg/100 g, which was comparable to the value of
leaves of Celery (Apium graveolens) 52.0 mg/100 g as reported by Gobezie et al. (1997). However, Gobezie et al. (1997) reported lower values than the present study for leaves of Cabbage (Brassica oleracea) 29.0 mg/100 g, Swiss chard (Beta vulgaris) 41.0 mg/100 g, Lettuce (Lactuca sativa) 31.0 mg/100 g and Yam (Dioscorea spp.) 13.3 mg/100 g. The mean value of the present study on Anchote leaves was lower than the values of Cauliflower (Brassica oleracea var. botrytis) 62.0 mg/100 g, Ethiopian kale (Brassica carinata) 64.0 mg/100 g and Spinach (Spinacea oleracea) 110.0 mg/100 g leaves (Gobezie et al., 1997). Ayalew (2016) also reported the mean value of phosphorus content 64.63 mg/100 g for the leaves of Anchote accessions. Phosphorus is an essential part of every living cell. It is involved in the enzyme-controlled energy-yielding reactions of metabolism. Phosphorus also helps to control the acid-alkaline reaction of the blood. Highest requirements for calcium and phosphorus are for the young, pregnant, and nursing mothers (Lyaka et al. 2014).

Iron contents in the accessions are listed in Table 4. The contents of Iron varied from 3.68 mg/100 g in “P4-Acc40” to 11.51 mg/100 g in “P97-Acc147”. The Iron content of accession “P97-Acc147” was significantly (P < 0.05) higher than all the accessions but this did not differ significantly (P < 0.05) from accession “P55-Acc111” (10.15 mg/100 g). Accession “P4-Acc40” had significantly (P < 0.05) lower (3.68 mg/100 g) Iron content than all accessions except “P3-Acc118” (4.88%) and “P10-Acc151” (5.21%) on dry weight basis. The mean Iron content value of the study was 7.19 mg/100 g which was comparable to leaves of Celery (Apium graveolens) 5.2 mg/100 g and Spinach (Spinacea oleracea) 5.9 mg/100 g (Gobezie et al., 1997). A lower value compared to the present study on Anchote leaves reported by Gobezie et al. (1997) for leaves of Cabbage (Brassica oleracea), Cauliflower (Brassica oleracea var. botrytis), Ethiopian kale (Brassica carinata), Swiss chard (Beta vulgaris), Lettuce (Lactuca sativa) and Yam (Dioscorea spp.). The values were 0.8 mg/100 g, 0.8 mg/100 g, 4.1 mg/100 g, 3.6 mg/100 g, 1.6 mg/100 g and 1.8 mg/100 g, respectively. Ayalew (2016) also reported the mean value of iron content 8.54 mg/100 g for the leaves of Anchote accessions. The importance of Iron in the body is its role in the hemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrates, protein and fats (Parmar et al., 2017). With a prolonged Iron deficiency, the hemoglobin falls below normal and the condition is known as anemia (Saha et al., 2015).

Zinc contents in the accessions are shown in Table 4. The content of Zinc varied between 1.02 mg/100 g in “P97-Acc147” and 3.11 mg/100 g in “P10-Acc151”. Zinc content of accession “P10-Acc151” was higher (3.11 mg/100 g) but this did not differ significantly from all accessions except “P4-Acc40” (1.11%), “P55-Acc111” (1.94%), and “P85-Acc5” (1.98%). Accession “P97-Acc147” had significantly (P < 0.05) lower (1.02 mg/100 g) Zinc content than all accessions except “P4-Acc40” (1.11%) and “P55-Acc111” (1.94%) on dry weight basis. The mean zinc concentration was 2.21 mg/100 g, which was lower than leaves of Brassica juncea (7.50 mg/100 g) and Chenopodium album (7.50 mg/100 g), and higher than Moringa oleiferal leaves (1.50 mg/100 g) as recorded in Saha et al. (2015) study. Ayalew (2016) also reported the mean value of zinc content 1.95 mg/100 g for the leaves of Anchote accessions. Zinc is an essential trace element, which functions as an integral part of numerous enzymes or as a stabilizer of the molecular structure of sub-cellular constituents and membrane. It also participates in the synthesis and degradation of carbohydrates, lipids, protein and nucleic acids (Saha et al., 2015). Zinc plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation, and wound healing (Gemede et al., 2016; Mliton et al., 2014).

5.4. Mineral ratios
The mineral ratios, which was calculated by dividing the first mineral level to the second mineral level (Gemede et al., 2016; Wang et al., 2014), are presented in Table 5.

6. Calcium to magnesium
Calcium (Ca) to Magnesium (Mg) ratio present in the accessions is shown in Table 5. The Ca to Mg ratio of the leaves of Anchote accessions ranged from 1.4 for accession P85-Acc5 to 3.2 for accession P4-Acc40. The mean Ca to Mg ratio was 2.1, which met the standard with a value greater than two
considered as a good mineral balance (Adeyeye et al., 2012). In general, the Ca to Mg ratio in the human body is referred to as the blood sugar ratio. Both minerals are very much interrelated in such a way that calcium is required for the release of insulin from the pancreas whereas magnesium inhibits insulin secretion. Magnesium is also necessary to keep calcium in solution (ARL, 2012).

4. Calcium to potassium
The Calcium (Ca) to Potassium (K) ratio present in the accessions is shown in Table 5. Ca/K ratios among the accessions ranged from 0.7 to 1.2. Accession P109-Acc162 showed the least ratio while P3-Acc118 had the highest ratio. The mean Ca/K ratio was 1.0, which met the standard, a ratio less than 4 considered as good source of the mineral balance. The Calcium to Potassium (Ca/K) ratio is called the thyroid ratio because calcium and potassium play a vital role in regulating thyroid activity (Gemede et al., 2016; Ojiezeh et al., 2016). Calcium is affected by several hormones and is considered to be under parasympathetic (an involuntary nervous system that serves to slow the heart rate, increase intestinal and glandular activity) control. The elevation of the Ca/K ratio can be indicative of reduced thyroid expression. In contrary, a low Ca/K ratio would indicate an elevation of thyroid expression (Watts, 2010). Watts (2010) also reported that the ideal ratio of Ca/K is 4:1 with an acceptable ideal range of 2.2 to 6.2. A higher Ca/K levels in foods is required for favorable calcium absorption in the intestine for bone formation (Gemede et al., 2016; Wang et al., 2014).

5. Calcium to phosphorus
The Calcium to Phosphorus ratio present in the accessions is listed in Table 5. The Ca to P ratio of the leaves of Anchote accessions varied from 1.3 for accessions P80-Acc201 and Acc109 to 2.1 for P55-Acc111 and presented in Table 6. The mean Ca to P ratio of the accessions was 1.7, which was much satisfactory compared to the standard, a ratio greater than 0.5 is a good mineral balance. The recommended Ca to P ratio should be greater than 0.5 (Gemede et al., 2016; Wang et al., 2014). Ca to P ratio greater than 2 also contributes to the absorption of calcium in the small intestine (Adeyeye & Aye, 2005; Oze, 2011). Furthermore, food is considered as good if Ca to P ratio is greater than 1 and poor if this ratio is less than 0.5 (Gemede et al., 2016; Oze, 2011). A higher calcium to phosphorous level in foods is required for favorable calcium absorption in the intestine for bone formation (Adeyeye et al., 2012). According to Ijarotimi et al. (2013), diets rich in protein and phosphorus may promote the loss of calcium in the urine. The Ca to P ratio in this study indicates that the leaves of Anchote accessions would help calcium absorption in the body. The

| Name of Accession | Ca:Mg | Ca:K | Ca:P | Na:K | Fe:Zn |
|------------------|-------|------|------|------|-------|
| P3 - Acc118      | 1.7   | 1.2  | 1.8  | 0.7  | 1.9   |
| P10 - Acc151     | 1.7   | 1.1  | 1.8  | 0.6  | 1.7   |
| P85-Acc5         | 1.4   | 0.9  | 1.4  | 0.6  | 4.1   |
| P55-Acc111       | 2.6   | 0.9  | 2.1  | 0.5  | 5.2   |
| P80-Acc201       | 2.4   | 0.9  | 1.3  | 0.6  | 2.3   |
| P90-Acc162       | 1.9   | 0.9  | 1.6  | 0.6  | 2.3   |
| P97-Acc147       | 2.8   | 0.8  | 1.8  | 0.6  | 11.3  |
| P109-Acc149      | 1.5   | 0.7  | 1.3  | 0.6  | 4.0   |
| P4-Acc40         | 3.2   | 0.9  | 2.0  | 0.5  | 3.3   |
| P93-Acc128       | 1.7   | 1.2  | 2.0  | 0.6  | 2.5   |
| Mean             | 2.1   | 1.0  | 1.7  | 0.6  | 3.9   |
| Standard         | >2    | <4   | >0.5 | <1   | >2    |

Note: Number of sample size is fifteen and replicates are three.
### Table 6. Calculated mineral safety index (MSI) values for accessions

| Name of Accessions | Calcium (mg/kg D) | Magnesium (mg/kg D) | Sodium (mg/kg D) | Phosphorus (mg/kg D) | Iron (mg/kg D) | Zinc (mg/kg D) |
|--------------------|------------------|---------------------|------------------|----------------------|---------------|---------------|
| P3-Acc118          | 0.76             | 9.24                | 2.03             | 12.97                | 0.49          | 4.31          |
| P3-Acc113          | 0.81             | 9.39                | 2.20             | 12.80                | 0.45          | 4.29          |
| P85-Acc5           | 0.78             | 9.22                | 2.29             | 12.71                | 0.56          | 4.24          |
| P55-Acc111         | 0.84             | 9.16                | 2.57             | 12.43                | 0.55          | 4.25          |
| P80-Acc162         | 0.67             | 9.33                | 1.45             | 13.55                | 0.5           | 4.25          |
| P90-Acc128         | 0.66             | 9.34                | 1.76             | 13.74                | 0.46          | 4.34          |
| P97-Acc149         | 0.72             | 9.28                | 1.55             | 13.45                | 0.65          | 4.15          |
| P10-Acc151         | 0.84             | 9.16                | 1.71             | 13.15                | 0.55          | 4.25          |
| P93-Acc128         | 0.69             | 9.31                | 1.55             | 13.80                | 0.65          | 4.15          |
| Note: Number of sample size is fifteen and replicates are three. | | | | | | |
high Ca to P ratio observed in this study is also of nutritional benefit, particularly for children and the aged who need higher intakes of calcium for bone formation and maintenance (Gemede et al., 2016; Ijarotimi et al., 2013).

### 6. Sodium to potassium

The Sodium to potassium ratio present in the accessions is shown in Table 5. The Na to K ratio of the leaves of Anchote accessions ranged from 0.5 to 0.7 with the mean ratio of 0.6, which was below the standard, a ratio less than one considered as a good mineral balance. The accessions P55-Acc111 and P4-Acc40 had the lowest ratio while P3-Acc118 gained the highest value. The recommended Na to K ratio should be less than one (Gemede et al., 2016; Wang et al., 2014). Ijarotimi et al. (2013) also reported that the Na to K ratio less than one is recommended for diets, particularly for hypertensive patients. Therefore, the observed Na to K mineral ratio showed that leaves of Anchote would help to prevent hypertension and might lower blood pressure in hypertensive patients. It may be also suitable for people who have the risk of high blood pressure. This ratio is also referred as the life-death ratio in humans physiology because it is so critical in their interdependence since the Sodium pump mechanism and the electrical potential of cells is regulated by Sodium and potassium levels (Gemede et al., 2016; Wang et al., 2014). Sodium is normally extracellular while potassium is normally intracellular. If the ratio of these minerals is unbalanced, it indicates important physiological malfunctions within the cells (ARL, 2012).

### 7. Iron to zinc

The Iron to zinc ratios of the leaves of Anchote accessions are presented in Table 6. The Fe to Zn ratio of the accessions varied from 1.7 for P10-Acc151 to 11.3 for P97-Acc147. The mean Fe to Zn ratio of the accessions was 3.9. Pérels et al. (2001) reported that Iron did not impair zinc absorption up to an Iron: zinc ratio of 2:1. Beside on this report, one can conclude that the Iron present in the accessions did not impair zinc absorption. Iron functions as hemoglobin in the transport of oxygen and in cellular respiration, as it functions as essential component of enzymes involved in carbohydrates, protein, and fat oxidation to control body weight, which is very important factor in diabetes (Moses et al., 2012). Vitamins A and E metabolism and bioavailability are dependent on zinc status (Ibrahim et al., 1999; Soetan et al., 2010).

### 8. Mineral safety index

All the calculated MSI values were less than standard MSI (Table 6) meant that none of the minerals would constitute mineral overload or became toxic to the leaves consumers. All the differences between the standard and calculated MSI values were positive which implied that the body might not be overloaded with the listed minerals (Nieman, 1992).

| Mineral Variables | PC1   | PC2   |
|-------------------|-------|-------|
| Calcium           | 0.203 | −0.651|
| Magnesium         | 0.210 | −0.596|
| Sodium            | 0.500 | 0.295 |
| Potassium         | 0.558 | 0.010 |
| Phosphorus        | 0.154 | 0.132 |
| Iron              | 0.428 | 0.314 |
| Zinc              | −0.383| 0.133 |
| **Eigen values**  |       |       |
| **Percentage variation** | 40.14 | 29.16 |
| **Cumulative variation** | 40.14 | 69.30 |

Note: Number of sample size is fifteen and replicates are three.
8.1. Principal component analysis of mineral traits

The principal component analysis (PCA) result of the mineral traits is listed in Table 7. The PCA showed that two Eigen vectors were required for reaching a total variance of 69.30% among the 10 Anchote accessions evaluated. The first component accounted for 40.14% of the total variation and the second contains 29.16%. PC 1 comprises five accessions, which are P90-Acc162, P80-Acc201, P93-Acc128, P3-Acc118, and P10-Acc151. PC 2 also consists of the other five accessions, which are P109-Acc149, P97-Acc147, P85-Acc5, P55-Acc111, and P4-Acc40.

Scatter plot of the accessions on PCs based on mineral traits showed that Cluster 1 contained the accessions P10-Acc151, P90-Acc162, P80-Acc201, P3-Acc118, and P93-Acc128, with high loading for zinc and low value for Sodium, potassium and Iron. Whereas cluster 2 consisted of accessions, which were P4-Acc40, P55-Acc111 and P85-Acc5, with high loading for calcium. P85-Acc5 had highest value for magnesium and phosphorus whereas iron (in P4-Acc40) was recorded as lowest value. The accessions P97-Acc147 and P109-Acc149 belong to the third cluster with highest loading for iron, sodium, and potassium and the lowest value in calcium, magnesium, phosphorus (P97-Acc147) and zinc (P97-Acc147) (Figure 2).

Principal component analyses (PCA) techniques identify plant traits that characterize the distinctness among selected genotypes (Kubie, 2013). As shown in Table 6, the nutrients affecting the PC1 were the high positive loading for potassium, sodium, and iron while the high negative loading for Calcium and Magnesium content and positive loading for Zinc affected PC2.

| Table 8. Cluster means of Anchote accessions based on mineral traits |
|---------------------------------------------------------------|
| **Mineral variables** | **Clusters** | **Cluster 1** | **Cluster 2** | **Cluster 3** |
| Calcium             |               | 86.23        | 98.36         | 83.67         |
| Magnesium           |               | 43.89        | 53.62         | 42.51         |
| Sodium              |               | 49.21        | 54.76         | 69.98         |
| Potassium           |               | 82.96        | 105.59        | 110.18        |
| Phosphorus          |               | 51.99        | 54.93         | 53.72         |
| Iron                |               | 5.86         | 7.30          | 10.33         |
| Zinc                |               | 2.76         | 1.68          | 1.65          |

Note: Number of sample size is fifteen and replicates are three.
The clusters mean values of the accessions are presented in Table 8. The first cluster comprises accessions (P10-Acc151, P90-Acc162, P80-Acc201, P3-Acc118, and P93-Acc128) that showed highest mean value for zinc (2.76 mg/100 g) whilst lowest mean value recorded for Sodium (49.21 mg/100 g), potassium (82.96 mg/100 g), phosphorus (51.99 mg/100 g) and Iron (5.86 mg/100 g). The second cluster consisted of accessions (P4-Acc40, P55-Acc111 and P85-Acc5) with the highest mean value in calcium (98.36 mg/100 g), magnesium (53.62 mg/100 gm) and phosphorus (54.93 mg/100 g) whereas no lowest mean value was recorded. The third cluster contained accessions (P97-Acc147 and P109-Acc149) with the highest value for Iron (10.33 mg/100 gm), Sodium (69.98 mg/100 gm) and potassium (110.18 mg/100 g) and the lowest values in magnesium (42.51 mg/100 gm) and zinc (1.65 mg/100 gm) were obtained.

8.2. Phytochemical contents

8.2.1. Condensed tannin content

The results of the condensed tannin content of the accessions evaluated are listed in Table 9. The condensed tannin content of the accessions ranged from 129.0 ± 6.89 mg/100 g to 255.32 ± 4.62 mg/100 g for the accessions P4-Acc40 and P10-Acc151, respectively. A significant difference (P < 0.05) was observed in between the ranges and P85-Acc5 had a significant difference (P < 0.05) with the rest of the accessions. The mean value of condensed tannin content was 189.72 mg/100 g. Ayalew (2016) also reported higher condensed tannin content of 216.53 mg/100 g for the leaves of Anchote accessions. According to Essack et al. (2017), tannins are plant polyphenols that are capable of forming complexes with metals ions and macromolecules like proteins and polysaccharides. Tannins affect the nutritional value of food products by chelating metals like Iron and zinc and reducing the absorption of these nutrients as well as forming complexes with protein thereby inhibiting their digestion and absorption (Olawoye & Gbadamosi, 2017). The antinutritional/toxicity effects of tannin depend upon their chemical structure and dosage (Fekadu et al., 2013). Therefore, the condensed tannin content of leaves of Anchote accessions obtained from this study may not lead to toxicity since the total acceptable tannic acid daily intake for a man is 560 mg/100 g and further reduced during traditional processing (Fekadu et al., 2013). Condensed Tannins have a wide range of biological and pharmacological activities including antioxidative, cardio-protective, antitumor, antibacterial, antiviral, anti-inflammatory and immune-modulatory. These protective effects are related to their capacity to: (a) act as free radical scavengers; (b) activate antioxidant enzymes (Kumari & Jain, 2012).

Means within the same column with different superscripts were significantly (P ≤ 0.05) different. Values are presented as Mean ± SE of replicate determinations n = 3 and the number of sample size is fifteen.

Table 9. Phytochemical compositions of five leaves of Anchote accessions (on dwb)

| Accessions name | Condensed Tannin (mg/100 g) | Phytate (mg/100 g) | Total Flavonoid (mg/g QE) | Total Phenol (mg/g GAE) |
|-----------------|-----------------------------|-------------------|--------------------------|------------------------|
| P97-Acc147      | 230.16 ± 6.87abc           | 203.17 ± 1.62abc  | 8.03 ± 0.11abc           | 19.84 ± 0.55abc        |
| P4-Acc40        | 129.00 ± 6.89abc           | 196.67 ± 2.44ab   | 10.32 ± 0.15bc           | 29.96 ± 0.48abc        |
| P85-Acc5        | 198.08 ± 2.32c             | 188.66 ± 0.81c    | 9.23 ± 0.36cd            | 18.43 ± 0.38c          |
| P10-Acc151      | 255.32 ± 4.62abc           | 206.62 ± 1.01ab   | 11.82 ± 0.63ab           | 21.53 ± 0.29b          |
| P3-Acc118       | 136.03 ± 4.65c             | 194.25 ± 2.03bc   | 12.74 ± 0.57ab           | 20.17 ± 0.12bc         |
| Mean            | 189.72                     | 197.87            | 10.43                    | 21.98                  |
8.2.2. Phytate contents
The phytate contents of the accessions evaluated are shown in Table 9. The range of phytate content in this study was from 188.66 ± 0.81 for accession P85-Acc5 to 206.62 ± 1.01 for accession P10-Acc151 in which a significant difference (P < 0.05) was observed. On this study, the mean value of the accessions was 197.87 mg/100 g. Ayalew (2016) also reported a much higher phytate content of 250.30 mg/100g for the leaves of Anchote accessions. Phytates are a naturally occurring phosphorus storage compound present in green leafy vegetables. It has been shown to inhibit and reduce the absorption as well as bioavailability of minerals and metal ions like zinc, iron etc. (Essack et al., 2017). Foods high in phytic acid are getting a bad reputation due to its ability to bind to essential minerals such as iron, zinc, calcium, and magnesium in the digestive tract and inhibit their absorption by the body (Nissar et al., 2017). Recent studies indicate despite being somewhat demonized for its ability to reduce mineral absorption, phytic acid actually has some potentially beneficial properties. On the plus side, phytic acid can act as antioxidant, exhibits anti-cancer properties, and may have a positive impact on cholesterol and blood sugar (Nissar et al., 2017; Onomi et al., 2004). Preparation methods can reduce the phytic acid content in food, as well as adjusting meal times and food choices that can help to have better mineral absorption (Sade, 2009).

8.2.3. Total flavonoids contents
The total flavonoids contents of the accessions evaluated are listed in Table 9. The total flavonoids content range was from 8.03 ± 0.11 mg/g QE for P97-Acc147 to 12.74 ± 0.57 mg/g QE for P3-Acc118 and there was a significant difference (P < 0.05) between the ranges. A significant difference (P < 0.05) between the highest (P3-Acc118) and the two lower (P97-Acc147 & P85-Acc5) were observed. The mean value was 10.43 mg/g QE that was lower than those reported by Nyonje (2015) for Amaranthus caudatus (69.67 mg/g QE). Mean and Mohamed (2001) examined the flavonoids content of 62 vegetables and found that broccoli, cauliflower, cabbage, Chinese cabbage, and kailan contained between 148 and 219 mg/kg offlavonoids.Flavonoids are a ubiquitous group of polyphenolic substances, which have antibacterial, anti-inflammatory, anti-allergic, anti-neoplastic, antiviral, anti-thrombotic, and vasodilatory activities (Kopsell et al., 2005). Good correlation between the total flavonoids content and antioxidant activity has been show indicating that the flavonoids contribute in free radical scavenging (Cushnie & Lamb, 2005; Nyonje, 2015).

8.2.4 Total phenol contents
The total phenol contents of the accessions evaluated are presented in Table 9. The total phenol content of the accessions ranged from 18.43 ± 0.38 mg/g GAE (P85-Acc5) to 29.96 ± 0.48 mg/g GAE for P4-Acc40 in which a significant difference observed. As presented in Table 11, there was a significant difference (P < 0.05) between P4-Acc40 with the rest. The mean value of the study was 21.98 mg/g GAE that was lower than total polyphenols in young pods of raw, cooked and sundried A. esculentus with the values as 55.7 mg/g GAE, 51 mg/g GAE and 51.7 mg/g GAE,

| Accessions name | Phy/Ca | Phy/Fe | Phy/Zn | [Ca]/[Phy]/[Zn] |
|-----------------|--------|--------|--------|-----------------|
| P97—Acc147      | 0.14   | 1.44   | 19.97 ± 2.58’a| 0.05 ± 0.01’a |
| P4—Acc60        | 0.12   | 4.54 ± 0.26’o| 17.48 ± 0.85’p| 0.05 ± 0.0’p   |
| P85- Acc5       | 0.12   | 1.98 ± 0.04’c| 9.4 ± 0.42’o | 0.03 ± 0.01’b  |
| P10- Acc151     | 0.13   | 3.39 ± 0.33’o| 6.87 ± 1.53’p| 0.02 ± 0.0’p   |
| P3- Acc118      | 0.13   | 3.38 ± 0.19’o| 7.37 ± 0.29’o| 0.02 ± 0.0’p   |
| Mean            | 0.13   | 2.96   | 12.22   | 0.03            |
| Critical value  | < 0.24 | < 1    | < 15    | < 0.5           |
respectively (Abera, 2014). Ayalew (2016) also reported a higher total phenol content ranged from 26.42–59.9 mg/g GAE for the leaves of Anchote accessions. According to the study by Ritter et al. (1992), when the total polyphenol content in all the vegetables tested measured, there was a significant inverse correlation with Iron absorption. A number of vegetables associated with low Iron absorption turned bluish-black when Iron added to them, suggesting that the total polyphenol content in them was high. Phenolic compounds are substances that possess an aromatic ring bearing a hydroxyl substituent, including their functional derivatives such as esters, methoxy compounds and glycosides (Park et al., 2018).

8.2.5. Bioavailability of minerals

8.2.5.1. Phytate: calcium molar ratio. The calculated Phy: Ca molar ratios are presented in Table 10. The Phy: Ca ratio ranges from 0.12 for accessions P4-Acc40 and P85-Acc5 to 0.14 for accession P97-Acc147 through which a significance difference (P < 0.05) was maintained in between the ranges. The mean Phy: Ca ratio value was 0.13 mol/g, which was lower than the reported critical molar ratio, indicating that absorption of calcium would not be affected by phytate in these leaves. Phytic acids markedly decrease Ca bioavailability and the Phy: Ca molar ratio has been proposed as an indicator of Ca bioavailability (Gemede & Fekadu, 2014; Latta & Eskin, 1980). The Phy: Ca molar ratios < 0.24 considered as indicative of good calcium bioavailability.

8.2.5.2. Phytate: zinc molar ratio. The calculated Phy: Zn molar ratios are shown in Table 10. In this study, the Phy: Zn ratio ranged from 6.87 ± 1.53 for P10-Acc151 accession to 19.97 ± 2.58 for P97-Acc147 accession. A significance difference (P < 0.05) between the ranges has been shown. There was significance difference (P < 0.05) observed in between P97-Acc147 and the rest of the all the accessions except with P4-Acc40. The accessions P85-Acc5, P10-Acc151, and P3- Acc118 with their respective ratio had a good bioavailability ratio <15 whereas P97-Acc147 and P4-Acc40 which had a ratio > 15 as an indicator of poor zinc bioavailability. In general, the mean value of the study was 12.22 mol/g. It indicates good zinc bioavailability (Gemede & Fekadu, 2014; Morris & Ellis, 1989).

The importance of foodstuff as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH (Gemede & Fekadu, 2014; Oberleas, 1983). Zinc deficiency has been shown to be the cause of dwarfism and hypogonadism among adolescents (Gemede & Fekadu, 2014; Prasad, 1984). Zinc has been described as the essential mineral most adversely affected by phytate and the phytate-to-zinc molar ratio has been proposed as an indicator of zinc bioavailability (Gemede & Fekadu, 2014; Plaami, 1997).

8.2.5.3. Phytate: iron molar ratio

The calculated Phytate: Iron molar ratios are presented in Table 10. The range of Phytate: Iron ratio was 1.4 for accession P97-Acc147 to 4.54 ± 0.26 for accession P4-Acc40, which implied that the absorption of Iron from the accessions would be inhibited by phytate and as a result, the bioavailability of Iron would be poor. Phytate begins to lose its inhibitory effect on Iron absorption when phytate: Iron molar ratios are less than 1.0, although even ratios as low as 0.2 exert some negative effect (Gemede & Fekadu, 2014; Hurrell et al., 1992). The phytate: Iron molar ratios >1 was regarded as indicative of poor Iron bioavailability (Siegenberg et al., 1991). A significance difference (P < 0.05) between the ranges was observed and there was a significance difference (P < 0.05) in between the accessions P97-Acc147 with the rest except P85-Acc5 and P4-Acc40 with all.

8.2.5.4. [Calcium][phytate]: [zinc] molar ratio. The calculated [Ca][phytate]/[Zn] mill molar ratios are presented in Table 10. In this study, the value ranges from 0.02 ± 0.00 for accessions P10-Acc151 and P3-Acc118 to 0.05 ± 0.00 for both accessions P4-Acc40 and P97-Acc147 in which a significance difference (P < 0.05) between ranges was observed. The [Ca][phytate]/[Zn] mill molar ratio which was <0.5 mol/kg for all the accessions showed good zinc bioavailability. In general, the mean value of the study was 0.03 mol/kg, which was lower than the critical level. This
indicates there was less phytate-induced decrease in zinc bioavailability due to the higher calcium content in the food. The potential effect of calcium on zinc absorption in the presence of high phytate intakes has led to the suggestion that the [Phy]/[Ca]/[Zn] molar ratio may be a better index of zinc bioavailability than the [Phy]/[Zn] molar ratio alone. High calcium levels in foods can promote the phytate-induced decrease in zinc bioavailability when the [Ca]/[phytate]/[Zn] molar ratio exceeds 0.5 mol/kg (Gemed & Fekadu, 2014; Gibson, 1994).

9. Conclusions
In conclusion, the results of this study revealed that there is a significant difference ($P < 0.05$) in proximate, mineral, and phenolic compositions of leaves of Anchote accessions except in moisture content in dwb. The most notable finding of this study is that leaves of Anchote accessions were found to be good source of vital nutrients like crude protein, crude fiber, calcium, iron and zinc, which are good for human and animal health. The study also revealed that leaves of Anchote could be good source of Phenolics. Moreover, those leaves of accessions that contained significantly higher amounts can be implemented for breeding, new product development, and supplementation programs. Therefore, leaves of Anchote contained vital nutrients and seemed to have the potential to contribute for food security and its promotion for consumption and cultivation should be encouraged. Further study needs to be carried out on the possibility of developing value-added products by itself or blending with other food ingredients.

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