



Comparative Study on Chemical Composition and Antioxidant Properties (GraphPad Prism Approach) of Wild Ethiopian *Z. spina-christi* and Indian *Z. jujube* Fruit Species

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Abstract

There are several underutilized and wild fruit species in Ethiopia that can be used as sources of human food consumption. This study aimed to examine total phenolic contents (TPC), total flavonoid contents (TFC), proximate, mineral contents, and the in vitro antioxidant potentials of the *spina-christi* (Ethiopian Kunkura/Gava) fruit as compared with Indian *jujube* (Ber fruits — Umaran, Gola, and Mundia) fruits. According to this study, Kunkura, Umaran, Gola, and Mundia fruits are important sources of minerals (Mg, K, Ca, Na, P, Fe, Zn, Mn, Cu, and Se) than common cereals and grains. Soxhlet methanolic extraction at 45 °C for about 4 h was used to extract the compounds from fruits. A new statistical method approach, known as GraphPad Prism software, was used for the estimation and evaluations of fruit extracts in terms of EC50 values for the first time. The antioxidant activity was measured by the commonly accepted assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]-diammonium salt (ABTS) while the EC50 values were ranged between 2.94 (Mundia, the minimum value) and 15.48 (Gola, the maximum value) mg/mL. The EC50 estimations of DPPH, FRAP, and ABTS free radical scavenging activities of the fruit extracts were performed using GraphPad Prism statistical model. The total phenolic contents found were 311.26 ± 0.63 mg GAE/100 g extract for Gola (minimum) and 354.71 ± 18.59 mg GAE/100 g extract for Umaran (maximum). The maximum and the minimum flavonoid contents were 151.69 ± 2.68 mg QE/100 g for Umaran, and 124.76 ± 0.07 mg QE/100 g for Kunkura, respectively. Therefore, these nutritionally rich fruits can be the potential raw material for the food industries involved in manufacturing of value-added products like jam, jelly, juice, additives, and other similar products.

Keywords Wild edible fruits · Proximate composition · Elemental content · Antioxidant potential · GraphPad Prism · EC50 values

Introduction

Wild edible plants are plant species that are neither cultivated nor domesticated, but exist in their natural environments and are used as sources of food and medicine (Beluhan and Ranogajec

2011; Lulekal et al. 2011). Padulosi et al. (2009) indicated underutilized plants as species whose potential has not been fully realized. From time immemorial, wild plants have been serving as an important source of food and income for the poor and malnourished children during famine and difficult times. They have made a substantial addition to the human diet, especially during times of war, failure, or other causes of food shortages. Recent findings showed that these wild edible plants are rich in chemical composition like protein, fat, dietary fiber, carbohydrate and energy (Serçe et al. 2010; Liu et al. 2012; Tee et al. 2014; Pereira et al. 2019), mineral composition and vitamins (FAO and WHO 2001; Bhat et al. 2010; Kuras et al. 2020; Sibiya et al. 2020), and bioactive compounds/phytonutrients (Delgado et al. 2014; Fu et al. 2015). Even if wild/underutilized edible fruits contribute significant amounts of nutrients and bioactive compounds, these are not utilized effectively in Ethiopia as well as in India.

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Although Ethiopian rural communities use these wild/underutilized edible plants as a means of survival especially during times of drought, famine, and other difficult times (Duguma 2020; Lulekal et al. 2011), there is no adequate research on the nutritional composition and health benefits of the plants. The extent of use of these wild/underutilized edible plants varies from region to region in the country due to lack of awareness, ecological differences, and cultural differences. Such a difference continues because of the lack of research and nutritional value awareness among societies. For instance, Godere (*Colocasia esculenta*) (Azene 2017), Yams (*Dioscorea* spp.) (Muluaem et al. 2018), and Anchote (*Coccinia abyssinica*) are the best examples, which are limited to the southern and some parts of the Oromia region, Ethiopia (Parmar et al. 2017). At the same time, some of the wild/underutilized plants in Ethiopia are widely available on the food table in other countries. A typical example in this regard is *Physalis peruviana* (Hassanien 2011; Ramadan 2011; Ahmed 2014), also known as golden berry in English and “awut” in Amharic. The same is true for Kunkura (*Z. spina-christi*), *Z. jujube/ujuba*. Dokma (*Syzygium guineense*), and Tiringo/Citron (*Citrus medica*), which are consumable in limited areas of the country.

The *Ziziphus jujube* and *Ziziphus spina-christi* fruit species belong to the Rhamnaceae family. These plants grow mostly in East Africa, some parts of Asia (India, China), and Australia (Gao et al. 2011; Singh et al. 2012; Du et al. 2013). *Ziziphus* species have more than 170 sub-species (Abdoul-azize 2016) including *spina-christi* (Kunkura/Gava — local name) and *jujube* (Umaran, Gola, Mundia — local name in India) species, which are important for human medicine (Asgarpanah 2012), nutrition, sources of unique flavor, antimicrobial treatments, and antioxidants (Adam 2019). There are more than 90 different *jujube*, *Z. mauritiana* Lam. (*Z. jujuba* L.) cultivars in India, which are adapted to warm climates (Pareek 2013; Doijode 2020). In India, the *jujube* fruit species are also called Ber fruits. The report of Rashwan et al. (2020) showed that *jujube* fruits contain very high amounts of bioactive compounds and, therefore, the fruit is consumed traditionally as functional food staffs in their wild states. In Italy and China, the *jujube* pulp was used for the preparation of some food products, such as jams, beverages, pickles, and jelly. In addition to these, the *Z. spina-christi* and *jujube* plant species are drought resistant; fruits are available in excess amounts and can be good sources of food in countries where these plant species grow, including in India and Ethiopia. As a result, studying nutrient composition and bioactive compounds is critical to increasing food supplements.

According to Pérez-Burillo et al. (2018), fruits are foods that contain plenty of vitamins, minerals, and bioactive phytochemicals like polyphenols. Even if these fruit species are the best sources of these nutrients and bioactive compounds, there are no reported studies in Ethiopia on nutritional composition, bioactive compounds, antioxidant potentials, and mineral contents of *spina-christi* fruits. Therefore, it is important to characterize the chemical composition and to produce a typical food product or an input for a food product as an additive from such fruits.

These fruits can be the important sources of the industry as the major inputs, since these fruits are nutritionally rich and the major sources of phytochemicals or as for one of the ingredients like, for instance, making jam, jelly, juice, additives, and others. These are also important in supporting the global food basket in minimally processed and processed food industries. Among many of processing industries, these fruits can be used for the development of functional or enriched foods or in nutraceuticals, pharmaceuticals, canned juice production, and cosmetics industries (Wang et al. 2018; Marmouzi et al. 2019).

Even though *Z. spina-christi* and *Z. jujube* fruit species have been in use for many years by herdsmen (cowherd or shepherd, goatherd) in tropical countries like in India and Ethiopia during drought or starvation, there is great dietary/nutrient and phytochemical composition importance of the fruits being as a staple food like other commonly consumed fruits. Every part of the *Z. spina-christi* and *jujube* plant (leaves, stems, root, and ash of the wood) is known for several medicinal applications (Shahat et al. 2001) and is a widespread traditional medicine, known also as *jujube* (having different species like Umaran, Golla, and Mundia) (Marmouzi et al. 2019). The *Z. jujubespina-christi* plant species has been used as both an edible fruit and medicine for a long time in India as well as in China (Wang et al. 2018; Marmouzi et al. 2019). Therefore, to investigate the chemical compositions of the fruits, total phenolic contents (TPC), total flavonoid contents (TFC), proximate, mineral contents, and the in vitro antioxidant potentials of the fruits need to be assessed. The antioxidant activities of any extracts are evaluated effectively by effective concentrations (EC50) (Wu and Ng 2008). EC50 values have previously been used to evaluate drug discoveries, drug performance, effects of fungicides on fungi and oomycete pathogens, dose–response relationship study of stimulus toxicity, environmental toxicants, and other similar activities and analysis (Huber and Koella 1993; Ewijk Jh 1993; Jiang and Kopp-schneider 2014; Noel et al. 2018). Nowadays, the applications are extending to functional/nutraceutical foods to see the health effects of the food or food products on humans (Chen et al. 2013; Suriyatem et al. 2017; Sridhar and Charles 2019).

Therefore, the study aimed to investigate TPC, TFC, proximate, mineral contents, and the in vitro antioxidant potentials of the *spina-christi* (Ethiopian Kunkura/Gava) fruit as compared with Indian *jujube* (Ber fruits — Umaran, Gola and Mundia) fruits. At the same time the new statistical method approach, known as GraphPad prism software, was used for the estimation and evaluations of fruit extracts in terms of EC50 values.

Materials and Methods

Raw Material

Ziziphus spina-christi (locally known as Kunkura/Gava) was collected from North Wollo, Ethiopia, in November

2018 whereas the three *Ziziphus jujube* fruit species (locally known as Umaran, Gola, and Mundia) were collected from Haryana, India, in January 2018 in the morning sessions. The geographical coordinates of the specific study areas are Raya-Kobo, Ethiopia, located at longitude: 39°37'59.9916"E and latitude: 12°9'0"N and Sonipat, India, at longitude: 75°42'20.52"E and latitude: 29°9'1.26"N. All fruit samples were collected at the medium maturity stage based on their accessibilities or convenient sampling method (Etikan 2016). In *jujube* and *spina-christi* fruit species, there are three maturities (white maturity, half-red maturity, and full red maturity) stages, but the first two maturity stages are rich in phenolics (TPC, TFC, and total phenolic acids) and the latter are rich in sugars and anthocyanins (Wang et al. 2016). The unripened fruits were selected, sorted out, and removed from the rest (the ripened) of the fruits. All the collected fruit samples were stored in a clean perfect (zipped) polyethylene flexible plastic sealed container (without oxygen transfer/exchange) and transported to the laboratory. Then, in the laboratory, the fruits were washed with tap water followed by distilled water and stored in a fruit storage fresh zone room with their clean plastic bags. Throughout the whole study period, the room temperature was between 20 and 25 °C.

Chemicals, Standards, and Equipment

All chemicals and reagents used in this study were of analytical grade and prepared by using high-purity deionized water obtained from a Milli-Q water purification system (Milli-Q Ultrapure Water System Corp., Bedford, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH, ≥97%), [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]-diammonium salt (ABTS, ≥97%), 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, ≥97%), gallic acid (98%), 2,4, 6-tripyridylS-triazine (TPTZ), and quercetin (≥97%) were obtained from Sigma-Aldrich India. Microwave digestion (Milestone, Ethos Easy), Kjeldahl (Buchi India Pvt. Ltd.), Automated Distillation Apparatus (Buchi India Pvt. Ltd.), ICP-OES (Optima 7000 DV, Perkin Elmer, USA), and UV-VIS spectrophotometer (SHIMADZU, UV-2600, USA) were used for analysis.

Extraction and Preparation of Extract Solution

The collected Kunkura fruit samples were transported to Addis Ababa Science and Technology University (AASTU), Industrial Chemistry Laboratory. Then, the fruit samples were washed with tap water followed by distilled water, and the edible parts of the fruits were removed from the seeds, and the edible flesh parts were dried at room temperature without exposure to sunlight. The air-dried edible portions of Kunkura fruits were transported to the National Institute of Food Technology, Entrepreneurship, and Management (NIFTEM), Haryana, India. The Umaran, Gola, and Mundia fruit species were

transported to NIFTEM and prepared similar to Kunkura fruit samples. After drying, each fruit sample was ground to powder, sieved with a 250-µm sieve, and stored in the refrigerator for further works.

The extraction for TPC, TFC, and antioxidant assay determination was performed according to the method stated by Alara et al. (2018) with some modifications. Accordingly, 10 g of the air-dried fruit samples was weighed on Whatman filter paper, rolled, stapled, and put into a Soxhlet extractor thimble. Using the optimized dried powder to methanol ratio (1:20 w/v), 200 mL methanol (boiling point 64.7 °C) was transferred to the thimble to make it wet for fast extraction. Each of the powdered samples was extracted continuously for 4 h at 40 °C using a heating mantle. The extract solutions were allowed to cool to room temperature and dried using a rotary evaporator. The extracts were kept at 4 °C for further studies.

Proximate Analysis

Proximate composition determination was done according to AOAC official method (AOAC 2000), Chew et al. (2011), and Konczak and Roule (2011). All proximate contents were measured in triplicate and method blanks were performed simultaneously in a similar manner with the samples. For moisture content (MC) determination, 5 g of fresh fruit sample was heated in a hot air oven at 105 °C for 4 h, cooled in desiccators, and weighted and the moisture content was calculated by the following formula (Eq. 1).

$$MC(\%) = \frac{(\text{Weight of fresh sample} - \text{Weight of dried})}{(\text{Weight of fresh sample taken})} \times 100\% \quad (1)$$

The ash content was determined by heating the samples dried for MC in a muffle furnace at 600 °C for 6 h and cooled in a desiccator and the weight of the ash was measured. Then, the ash content was calculated as follows (Eq. 2).

$$\text{Ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100\% \quad (2)$$

The crude fat content was determined by taking 1–5 g of fresh fruit samples and extracted with petroleum ether (boiling point 60–80 °C) using the Soxhlet apparatus for 6 h. The weight of the flask plus the extracted residue was measured after drying using a rotary evaporator and cooling in desiccators. Then, the fat content was determined by subtracting the empty flask from the flask containing fat residue multiplied by 100 (Eq. 3).

$$FC(\%) = \left[\frac{Fsk_{resid} - EFsk}{Wt \text{ of Sample}} \right] \times 100 \quad (3)$$

where *FC* is the fat content, *Fsk_{resid}* is the flask containing fat residue, *EFsk* is the empty flask, and *Wt of Sample* is the weight of sample.

Crude protein was determined by Kjeldhal method by taking 1 g of the sample digested with 20 mL concentrated H₂SO₄ and Kjeldhal catalyst (4 parts of K₂SO₄ and one part of CuSO₄) in a digestion block until it changes from greenish to clear and colorless solution. The blank control sample was digested parallel with the sample. After digestion, the clear sample solution was distilled in Kjeldhal distillation unit in the presence of 40% NaOH and 4% of boric acid in the receiver flask. The condensed ammonia was titrated against 0.2 N of H₂SO₄ after the addition of 3 drops of the mixture of methyl red and bromocresol indicator. The concentration of nitrogen and protein were calculated as follows (Eq. 4):

$$\%N = \frac{(A - B) \times N \text{ of } H_2SO_4 \times 0.014}{\text{Weight of sample}} \times 100 \quad (4)$$

where *A* is the volume (mL) of (0.2 N) H₂SO₄ used in sample titration, *B* is the volume (mL) of (0.1 N) HCl used in blank titration, and 14 is the atomic weight of nitrogen.

The content of protein was estimated by the formula: **protein(%) = %N × 6.25**, where 6.25 is the protein conversion factor.

Carbohydrate was calculated by difference from the formula: Carbohydrate (%) = 100 – [M.C. – Ash – Protein – Fat – dietary fiber] (Wisker and Feldheim 1990).

Mineral and Heavy Metal (Elemental) Contents

All glassware and polyethylene sample bottles were washed with 5 N nitric acid and de-mineralized water after soaking for 3 days with detergents. Mineral and heavy metal analyses were performed according to FDA 2010 and other related methods (Bhat et al. 2010; William 2010; Konczak and Roule 2011; Karasakal 2021) for microwave digestion and ICP-OES elemental analysis. Samples were digested with concentrated nitric acid in a microwave digestion apparatus. In detail, 0.5 g of dried fruit sample was placed in TFM vessel in safety hood and the mixture of HNO₃ and H₂O₂ (8:2) was transferred into the vessel. The mixture was digested at 280 °C and 850 Watt microwave power. The analysis was performed in triplicate for each sample. Method blanks were digested similarly with the sample. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be LOD (for method — MDL) = $\bar{X} + 3S_{\text{blank}}$ and LOQ = $\bar{X} + 10S_{\text{blank}}$, respectively, where *S*_{blank} is the standard deviations of the blank (*n* = 7) and 3 and 10 are the Student *t*-test values at 95% statistical confidence level (Miller and Miller 2010).

An ICP-OES was used for the measurement of Fe, Mn, Zn, P, Se, Cr, Cu, Na, K, Ca, Mg, and Pb. Standard calibration curves were prepared for all minerals and heavy minerals on the same day of analysis. This instrument was set with a

shear gas system, adjustable and quick-change torch cassette, cyclonic Spray Chamber, and two high-performance solid-state detectors (SCD). The ICP OES operating conditions were as follows: purge gas flow rate was high, delay time was 19 s, plasma condition was varying depending on each element, the sample flow rate was 1.5 mL/min, and flush time was 5 s.

Determination of Total Phenolic, and Total Flavonoid Contents and the Free Radical Scavenging Potential

Total Phenolic Content

The TPC of the four fruit extracts was determined by UV–VIS spectrophotometer using Folin–Ciocalteu reagent method (Alara et al. 2018). Briefly, 1 mL of extract at a concentration of 1 g/L was mixed with 200 μL of Folin–Ciocalteu reagent, which was diluted with methanol (1:10 ratio, respectively). Then, after 5 min 0.6 mL of 0.2 mM Na₂CO₃ solution was thoroughly mixed with the prepared mixture. The mixture solution was left for 130 min and absorbance was measured at 765 nm using a UV–VIS spectrophotometer. Finally, the concentration of TPC in the fruit extract was calculated from the gallic acid standard calibration curve (ranging from 50 to 400 mg/L, mg GAE/g dw Eq. 5) with absorbance at 765 nm. The control samples were performed using methanol instead of sample extracts with the same treatments as a sample.

$$TPC = \frac{C \times V}{m} \times DF \quad (5)$$

where *C* is the concentration obtained from calibration curve, *V* is the final volume, *m* is the mass of the fruit powder, and *DF* is the dilution factor.

Total Flavonoid Content (TFC)

For TFC determination, an aliquot of 400 μL of 1 g/L extract solution was thoroughly mixed with 400 μL of 2% AlCl₃ solution and allowed to stand for 30 min in the dark at room temperature. The absorbance of the clear-blue solution was measured at 417 nm using a UV–VIS spectrophotometer. Then, the concentration of TFC in the extract was calculated from the standard calibration curve (mg QE/g dw, following Eq. 6) of quercetin ranging from 50 to 500 mg/L (Alara et al. 2018).

$$TFC = \frac{C \times V}{m} \times DF \quad (6)$$

where *C* is the concentration obtained from the calibration curve, *V* is the final volume, *m* is the mass of the fruit powder, and *DF* is the dilution factor.

Scavenging Activity of 2,2-diphenyl-1-picrylhydrazyl Radical (DPPH)

In the presence of an antioxidant, which can donate an electron to DPPH, the purple color typical of free DPPH radical decays. The antioxidant scavenging activities of the fruit extracts were determined based on the scavenging effect on the stable DPPH free radical activity. Briefly, the DPPH stock solution was prepared by dissolving 1.9716 mg DPPH in methanol in a 50 mL brown volumetric flask and kept in the refrigerator till use. The absorbance was adjusted to 1.1 ± 0.05 by adding methanol or stock solution of DPPH in each day of measurement. In 15 mL amber vials, 2850 μ L DPPH solution was added into 150 μ L of each diluted fruit extract or trolox series standards (0.025 and 0.9 mM). After shaking properly, the mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 517 nm against a blank. Methanol was used as a method blank. The following equation (Eq. 7) was used to determine the percentage of the radical scavenging activity of each extract (Ilahy et al. 2011; Thaipong et al. 2006)).

$$\text{Scavenging effect(\%)} = \frac{(A_c - A_f)}{A_c} \times 100 \quad (7)$$

where A_c is the absorbance of the control/blank and A_f is the absorbance of the fruit extract.

Scavenging Effect of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]-diammonium salt (ABTS)

ABTS radical cation ($\text{ABTS}^{\bullet+}$) was generated by dissolving equal proportions of the reaction of a 7 mM ABTS (360.234 mg of ABTS in methanol in 100 mL volumetric flask) solution and 2.45 mM potassium persulfate (66.23 mg was dissolved with distilled water in 100 mL volumetric flask) solution. The mixture solution was stored in the dark at room temperature for 12 h before use. The $\text{ABTS}^{\bullet+}$ solution was diluted with methanol or $\text{ABTS}^{\bullet+}$ solution to adjust to an absorbance of 1.0 ± 0.02 at 734 nm. After the addition of 150 μ L of fruit extract or trolox (standard) to 10 mL size amber vials, 2850 μ L of $\text{ABTS}^{\bullet+}$ solution was added and the absorbance was measured at 734 nm after 4 min in the dark-room. The trolox standard calibration curve was constructed within 0.05–0.90 mM ranges and the percentage of the radical scavenging activity of each extract was calculated by the following equation (Eq. 8) (Thaipong et al. 2006).

$$\text{Scavenging effect(\%)} = \frac{(A_c - A_f)}{A_c} \times 100 \quad (8)$$

where A_c is the absorbance of the control/blank and A_f is the absorbance of the fruit extract.

Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP was performed using a method formerly described by Thaipong et al. (2006) with slight modifications. The fresh FRAP reagent was prepared in 500 mL brown bottles by mixing 250 mL of acetate buffer (300 mM pH 3.6), 50 mL of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) (10 mM), and 50 mL of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (50 mM). After the addition of 150 μ L of fruit extract or trolox (standard) to 10 mL size amber vials, 2850 μ L of FRAP solution was added. Then, the mixture solution was heated at 37 °C and allowed to react for a few minutes and the UV–VIS spectrophotometer measurement was performed at 593 nm. The trolox standard was used to draw a standard curve (0.025 and 0.7 mM) and is expressed as mM TEAC/g dry mass.

Effective Concentration (EC50)

The EC50 estimations of DPPH, FRAP, and ABTS free radical scavenging activities of the fruit extracts were done using Graph-Pad Prism statistical model. This statistical model was used following the first approach by Chen et al. (2013) and later by Sridhar and Charles (2019). The EC50 estimation was performed using non-linear Log (inhibitor) versus normalized response–variable slope curve fitting model. This model is estimating EC50 values within narrow ranges of maximum and minimum points using the following formula adopted from Chen et al. (2013) and later by Sridhar and Charles (2019) (Eqs. 9 and 10):

$$Y = \left[\frac{1}{1 + 10^{(\log \text{EC}_{50} - \log(X)) \times \text{Hillslope}}} \right] \quad (9)$$

where Y is representing the response activities of fruit extracts against in vitro antioxidants, X is the concentrations of fruit extracts, and Hillslope is a parameter to describe the steepness of the curve.

$$Y = \left[\frac{\text{Top} - \text{Bottom}}{(1 + 10^{[\log x_b - \log x] \times \text{Hillslope}})^S} \right] \quad (10)$$

where bottom asymptote is the baseline, top asymptote is the response (inhibition of the activity of fruit extracts), Hillslope is the parameter to describe the steepness of the curve, x_b is the concentration of the sample at the inflection point, and S is the asymmetry of the curve.

Statistical Analysis

All experiments were performed in triplicate and the results were expressed as the means \pm standard deviation (SD). The data were analyzed (one-way ANOVA, Tukey's HSD, post hoc descriptive statistics) using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows version 26). All the graphs in this study were analyzed by Excel Windows 10, GraphPad Prism 8.0.2.263, and OriginPro. 9.0 32 and 64 Bit.

Results and Discussion

Proximate Analysis

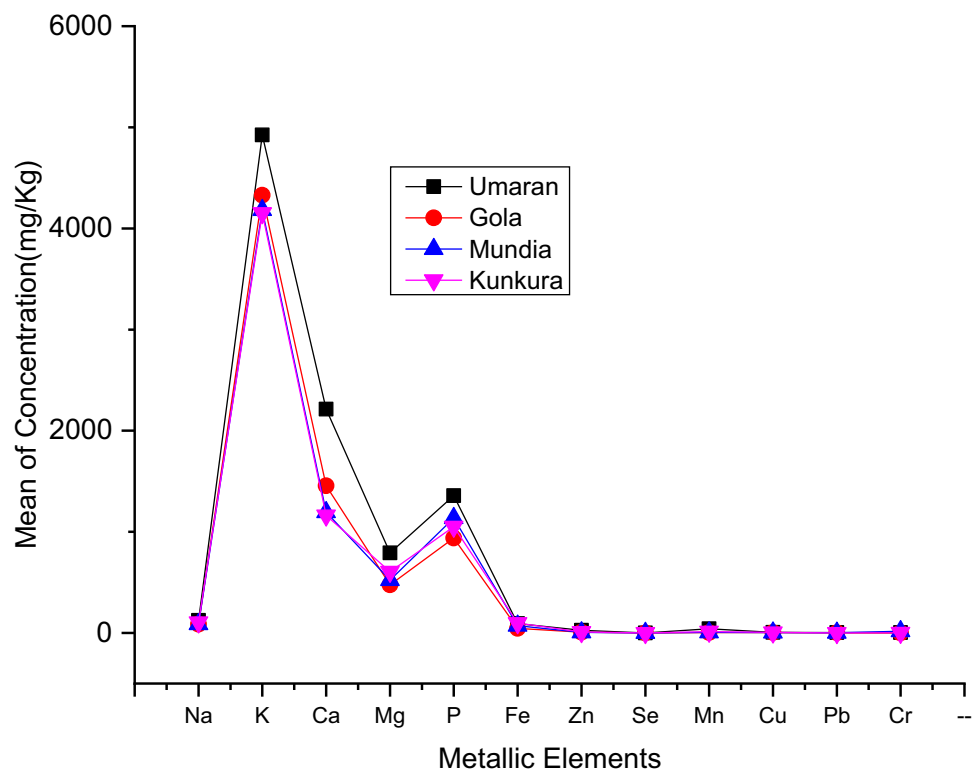
The proximate compositions of all four fruit samples namely Kunkura, Umaran, Gola, and Mundia fruits are presented in Appendix Table 5. The moisture content was the lowest ($81.77 \pm 5.74\%$) in Mundia fruit while the maximum ($88.22 \pm 0.10\%$) was found in Umaran on a wet basis (based on the fresh fruits). The lowest fat content was found ($0.81 \pm 0.02\%$) in Kunkura fruit while the highest ($8.84 \pm 0.16\%$) was in Gola fruit among others. After drying at room temperature, the moisture contents of Kunkura, Umaran, Gola, and Mundia fruits were determined and found to be 9.47 ± 1.41 , 10.03 ± 0.83 , 9.79 ± 0.17 , and $11.72 \pm 0.056\%$, respectively. The maximum (targeted) moisture content of dry fruits to ensure safe storage for a long time is 20%; therefore, the obtained results have lower water activities (Afolabi 2014). These MCs (after drying at room temperature for 1 week) of all fruits were in line with the previously reported results from Oman ($13.30 \pm 0.16\%$) (Singh et al. 2012) and Morocco ($13.96 \pm 0.22\%$) (El Maaiden et al. 2020), but higher than the reported data from Gusau, Zanfara, Nigeria ($3.13 \pm 0.67\%$) (Abubakar 2021). However, the reported data from China (Li et al. 2007) *jujube* species (18.99 ± 1.23 , 20.98 ± 1.12 , 17.38 ± 1.21 , 21.09 ± 1.39 and $22.52 \pm 1.43\%$) were higher than the current moisture contents. The protein content was highest ($1.32 \pm 0.04\%$) in Kunkura fruit and

lowest ($0.55 \pm 0.04\%$) in Umaran fruit as shown in Table 5. The carbohydrate and dry matter (DM) contents (calculated by differences) were ranged from 6.47 ± 3.63 to $10.65 \pm 1.96\%$ and 11.78 ± 0.10 to $18.89 \pm 3.60\%$. The highest carbohydrate and DM contents were obtained from Kunkura fruit while lower contents were found in other fruit species. There were no significant differences between fruit types for DM and carbohydrate contents. The values found in this study are similar to the reported values in the literature for Kunkura fruit (Osman and Ahmed 2009) and *jujube* fruits (Pareek 2013). Generally, there are significant differences ($\alpha=0.05$) in all samples within and between groups in all parameters. There were no significant differences ($p > 0.05$) among Umaran, Gola, and Mundia for both MC and ash contents. There are significant differences between the mean values of MC, ash, carbohydrate, and dry contents in Kunkura fruit samples compared with other fruit samples (Table 5). In general, all analyzed fruits had similar or closer proximate values as compared with Chinese *jujube* cultivars reported by Li et al. (2007). The analysis results of this study indicated that these underutilized fruits are rich in nutrient compositions.

Mineral and Trace Elemental Content Analysis

Potassium (K) is the most abundant mineral in all fruits (Fig. 1). Kunkura fruit was the lowest in K (4151.78 ± 1.64 mg/kg), and Umaran fruit was the highest (4924.75 ± 3.05 mg/kg). Generally, the highest K content was detected in all fruit

Fig. 1 Distribution of minerals for four *Ziziphus* fruit species from Ethiopia and India



samples compared with the other elemental contents. There were significant differences (at p value ≤ 0.05) between each pair of all fruits for K. Potassium levels in all fruits exceed the maximum safe limit for processed food set by FAO/WHO (550–1400 mg/kg) (FAO and WHO 2004). All fruits have high amounts of Ca (1162.80 ± 2.51 – 2212.50 ± 0.43 mg/kg) (Fig. 1). The contents of Na (106.03 ± 0.05 mg/kg), P (1051.37 ± 2.28 mg/kg), and Pb (BDL — below detection limit) in Kunkura fruits were lower than the reference standard limits set by WHO/FAO (Fig. 1). This study revealed that wild/underutilized edible fruits, commonly used since ancient times, can serve as a good alternative to improve the daily human intake of minerals. Similar high results were reported in 11 selected Southern Africa wild edible fruits (6902–14,271 mg/kg) by Sibiya et al. (2020).

Fe and Zn contents in all fruits were found to be high and sufficient for human nutrient balances (FAO and WHO 2004). The content of Zn (27.19 ± 0.07 mg/kg) in Umaran was much higher than the other fruit species. All the fruit species are good sources of functional and antioxidant mineral Zn for human consumptions. According to WHO/FAO report, Zn has a very important function in the protection of body tissues against oxidative stress and is helpful for antioxidant purposes in the human body. Mn (41.38 ± 0.25 mg/kg) content in Umaran fruit sample is much higher than the other Ethiopian and Indian fruit species (4.70 ± 0.05 – 13.39 ± 1.10 mg/kg). The levels of Cu, Pb, and Cr in all fruits were found to be above the safe limits of WHO/FAO/European Commissions; especially in Mundia fruit, Cr (17.73 ± 0.61 mg/kg) content was significantly high (Fig. 1). As a result, according to the current study, these minerals (Cu, Pb, and Cr) may have harmful effects on the consumers at these sampling points. Generally, Cr content was the lowest in Gola fruit followed by Kunkura fruit. Cu was the lowest in both Gola and Mundia fruits, but the highest in Kunkura and Umaran fruits.

Se content in Kunkura fruit was found to be 0.33 ± 0.04 mg/kg, dry weight basis, which is lower in comparison with world standards set by WHO/FAO. But Se was not detected in all other fruit species (Umaran, Gola, and Mundia). According to WHO/FAO report, selenium has a very important function in the protection of body tissues against oxidative stress, maintenance of defenses against infection, and modulation of growth and development (FAO and WHO 2004).

The method validation and quality control were performed using reference standards and blank parallel with the sample. The method was validated and the recoveries of the elements were within the accepted ranges (89–107%). The relative percent differences of matrix spike and matrix spike-dup were below 7.3% indicating that the method used was fairly precise.

Significant differences ($p > 0.05$) were not observed between Gola and Mundia for Na and Cu contents and between Gola and Kunkura for Cr content. However, all other elements significantly vary among all fruits. Additionally,

there were significant differences ($p \leq 0.05$) between groups and within groups for all fruit species (Fig. 1).

Estimated EDI of Minerals and Heavy Minerals

The estimated daily intake (EDI) of twelve minerals (Pb, Cr, Mn, Cu, Fe, Zn, Se, Ca, Mg, P, Na, and K) was calculated (Rahman et al. 2020) according to the mean concentration of each metal in each fruit and the respective consumption rates (Table 1). The EDI and maximum tolerable daily intake (MTDI, not reported by WHO/FAO for all minerals) of the studied minerals from consumption of fruits are shown in Table 1. Total daily intakes of some of the elements, which have the available MTDI from WHO/FAO in mg/day, are below the set values for all fruits. In all fruits, mean values of EDI decreased in the following order with the same fashion: $K > Ca > P > Mg > Na > Fe > Mn > Zn > Cu > Cr \approx Pb > Se$ (Table 1).

Total Phenolic, and Total Flavonoid Contents and In Vitro Antioxidant Analysis

The TPC results varied from 311.26 ± 0.63 mg GAE/100 g extract of Gola fruit, 332.42 ± 3.76 mg/100 g (Mundia), 344.84 ± 9.25 mg/100 g (Kunkura) to 354.71 ± 18.59 mg GAE/100 g extract of Umaran fruit (Table 2). The fruits had the highest contents of TFC in Umaran fruit (151.69 ± 2.68 mg QE/100 g), and the lowest was in Kunkura fruit (124.76 ± 0.07 mg QE/100 g). The TPC on the methanolic extracts of four fruits were found in the increasing order of $Gola > Mundia > Umaran > Kunkura$, whereas the contents of TFC were in the order of $Kunkura > Gola > Umaran > Mundia$ (Table 2). These variations may be due to Indian and Ethiopian geographical differences in general and climate differences such as attitude, soil type, and climate of the specific sampling areas in particular (Arruda et al. 2017). In the case of TPC, there were no statistically significant differences between Umaran and Kunkura, and Gola and Mundia, but there were significant differences between Umaran and Gola, Umaran and Mundia, Kunkura and Gola, and Kunkura and Mundia. Similarly, there were significant differences between Umaran and Gola, Umaran and Mundia, Umaran and Kunkura, Gola and Mundia, and Gola and Kunkura for TFC tests. In another way, between Mundia and Kunkura was not significantly varied.

The FRAP values were 279.95 ± 31.77 (Gola), 518.45 ± 31.57 (Mundia), 679.54 ± 2.71 (Umaran), and 760.44 ± 31.24 (Kunkura) mM trolox equivalent for antioxidant capacity (TEAC)/100 g dw in methanolic extracts at maximum inhibition ($> 90\%$ RSA, Table 2). The antioxidant capacity evaluated by ABTS radical scavenging assay were 129.09 ± 1.27 (Mundia), 151.77 ± 2.8 (Umaran), 151.77 ± 1.26 (Gola), and 153.85 ± 2.54 mM TEAC/100 g dw (Kunkura). In

Table 1 Estimated essential and non-essential elements of dietary intake of four different fruit species

Elements	MC				BW	DFC	EDI=DFC*MC/BW (mg/kg/day)				
	Umaran	Gola	Mundia	Kunkura			Umaran	Gola	Mundia	Kunkura	MTDI
Na	124.47	86.24 ^a	86.94 ^a	106.03	65	400	0.77	0.53	0.54	0.65	
K	4924.75	4328.56	4182.69	4151.78	65	400	30.31	26.64	25.74	87.09	
Ca	2212.5	1456.15	1194.49	1162.8	65	400	13.62	8.96	7.35	7.16	
Mg	791.67	474.96	523.04	605.88	65	400	4.87	2.92	3.22	3.73	
P	1357.54	937.85	1136.73	1051.37	65	400	8.35	5.77	7.00	6.47	
Fe	92.53	44.90	72.51	98.07	65	400	0.57	0.28	0.45	0.60	
Zn	27.19	7.29	4.9	9.43	65	400	0.17	0.04	0.03	0.06	60
Se	MDL	MDL	MDL	0.33	65	400	0.00	0.00	0.00	0.00	
Mn	41.38	4.7	5.17	13.39	65	400	0.25	0.03	0.03	0.08	2–5
Cu	5.63	3.39 ^b	3.95 ^b	6.35	65	400	0.03	0.02	0.02	0.04	30
Pb	4.07	2.52	2.96	0	65	400	0.03	0.02	0.02	0.00	0.21
Cr	4.06	0.53 ^c	17.73	1.15 ^c	65	400	0.02	0.00	0.11	0.01	0.2

EDI=DFC*MC/BW = where, BD=body weight, DFC=daily fruit consumption, AC=average metal concentration of metal in fruit samples. Letters with the same superscript in the same row are not significantly different at $p \leq 0.05$, unless otherwise there are significant differences between and among all elements and fruits. According to Joint FAO-WHO, the daily fruit consumption rate for an adult having a bodyweight of 65 kg was taken as 400 g minimum fruit and vegetable consumption. The estimated daily intakes (EDI) for the analyzed minerals were calculated by multiplying the mean concentration of the minerals determined in the fruit samples by the weight of fruits consumed by an average individual (Rahman et al. 2020)

the DPPH assay, the antioxidant capacity were 109.59 ± 5.21 (Mundia), 113.82 ± 1.81 (Umaran), 119.53 ± 4.47 (Gola), and 133.89 ± 3.86 (Kunkura) mM TEAC/100 g dw. The findings of this study were higher than the report of Pareek (2013) ($34.2\text{--}117.3$ mmol/100 g), Li et al. (2007) ($34.2 \pm 45.8\text{--}117.3 \pm 112$ mmol/100 g), and Wang et al. (2016) ($0.1\text{--}4.5$ mmol/100 g) of Chinese *jujube* fruit.

The statistical significant tests ($p \leq 0.05$) were performed among fruit samples and types of assays used (Table 2). There were statistically significant differences at $p \leq 0.05$ value between Umaran and Kunkura, Gola and Kunkura, and Mundia and Kunkura, but there were no significant differences between Umaran and Gola, and Umaran and Mundia in DPPH assay. In ABTS assay, there were significant differences ($p \leq 0.05$) between Umaran and Mundia, Gola and Mundia, and Mundia and Kunkura ($p > 0.05$), whereas there were no significant differences observed between Umaran and Gola, Umaran and Kunkura, and Gola and Kunkura. There were significant differences between Umaran and Gola, Umaran and Mundia, Gola in FRAP assay.

However, there were no significant differences between Umaran and Kunkura, and between Mundia and Kunkura for FRAP assay.

All of the fruit extracts were found to be capable of scavenging DPPH, ABTS, and FRAP radicals in a concentration-dependent manner, which shows that all of the fruit extracts have strong scavenging activity with respect to TEAC (Table 2). A similar study has been reported by Gao et al. (2011) in which DPPH (3794.5 ± 428.9 mmol TEAC/100 g much higher than this study values), ABTS (1.7 ± 0.3 mmol TEAC/100 g — which is much lower than this study values), and FRAP (626.0 ± 92.0 mmol TEAC/100 g — higher than this study values) assays have been assessed. In addition, the Oman *spina-christi* fruits were evaluated against these antioxidant potentials at a single point evaluation and the inhibition was from 87 to 98%. These study values were investigated (averagely from 5 to 99%) at different levels of fruit (at least 7 levels of concentration) and standard concentrations (9 points calibration). Not much comparison was made on these species, especially on Kunkura fruit,

Table 2 Antioxidant capacity, TPC, and TFC values (replicate measurements, $n=3$) of fruits

Fruit	TEAC (mM/100 g)			Total bioactive compounds (GAE/100 g dw)	
	DPPH	ABTS	FRAP	TPC (GAE/100 g dw)	TFC (QE/100 g dw)
Umaran	113.82 ± 1.81^a	151.77 ± 2.8^b	679.54 ± 2.71^c	354.71 ± 18.59^b	151.69 ± 2.68^c
Gola	119.53 ± 4.47^a	151.77 ± 1.26^b	279.95 ± 31.77^a	311.26 ± 0.63^a	145.74 ± 0.41^b
Mundia	109.59 ± 5.21^a	132.63 ± 2.87^a	518.45 ± 31.57^b	332.42 ± 3.76^a	127.08 ± 0.15^a
Kunkura	133.89 ± 3.86^b	153.85 ± 2.54^b	760.44 ± 31.24^d	344.84 ± 9.25^b	124.76 ± 0.07^a

The concentrations of DPPH, ABTS, and FRAP assays are based on the % radical scavenging activity results at maximum values (>90%). Letters with a different superscript in the same column are significantly different at $p < 0.05$

due to the lack of reported data in Ethiopia. According to these experimental results and previously reported data (Gao et al. 2011), *jujube* and *spina-Christi* fruit species are generally recognized as an outstanding source of biologically active compounds related to both nutritional and functional/nutraceutical values.

Effective Concentration (EC50)

The estimation of EC50 values of DPPH, ABTS, and FRAP radical scavenging activities of four fruit methanolic extracts was calculated using GraphPad Prism software model and is presented in Table 3. The Soxhlet methanol extract of all fruits had the highest inhibitory activity against DPPH, ABTS, and FRAP radicals as compared with EC50 estimation values of TEAC (Table 3). The tests were performed using 8 points of extract concentrations by decreasing the concentrations of each fruit extract (40, 20, 15, 8, 4, 0.8, 0.4, 0.2 mg/g or mg/mL of extracts of each fruit). The scavenging activities of fruit extracts against EC50 values of DPPH were found to be 2.94 mg/mL (Mundia), 3.09 mg/mL (Umaran), 3.56 mg/mL (Gola), and 3.68 mg/mL (Umaran) with a decreasing order. The EC50 values of fruits against ABTS were found to be 3.26 mg/mL (Mundia), 5.71 mg/mL (Umaran), 6.07 mg/mL (Kunkura), and 7.75 mg/mL (Gola). In the case of FRAP test, the EC50 values of fruits were found to be 11.89 mg/mL (Umaran), 13.28 mg/mL (Mundia), 15.33 mg/mL (Kunkura), and 15.48 mg/mL (Gola) with a decreasing order. The highest EC50 values were recorded against DPPH scavenging activities as compared with ABTS and FRAP methods (Table 3).

The lowest EC50 values (Table 3) revealed that higher antioxidant activities were observed in all fruits, which were an indication of the potential of the fruits for healthy consumption. There were significant differences among all fruits for both DPPH and FRAP antioxidant tests whereas in the case of ABTS test, there were significant differences among Umaran, Gola, and Mundia as well as among Kunkura, Gola, and Mundia. But there was no significant difference between

Umaran and Kunkura fruits for ABTS test. There is no similar study and comparison for these fruit extracts in the case of antioxidant EC50 values as far as our knowledge is concerned. There was also no similar study and comparison for these fruit extracts in the case of antioxidant %RSA values with different concentrations.

The designed model proposed by Chen et al. (2013) and Sridhar and Charles (2019) to estimate EC50 values of bioactive compounds extracted from fruits (Fig. 2) was effective in this experiment. As shown in Fig. 2, EC50 were evaluated from multiple experiments (7–9 points) and which was a simple and robust method to estimate the effective concentrations of fruit antioxidant compounds. From normalized sigmoid curve (Fig. 2) from the concentration–response curve of fruit extracts, the fruit extracts showed strong association between concentration and percentage inhibition against DPPH, ABTS, and FRAP radicals. The obtained normalized sigmoid curve (Fig. 2) for all fruit samples was similar to the reported sigmoid curve by Sridhar and Charles (2019) for Kyoho grape extracts in DPPH and ABTS assays.

In general, the optimum values of proximate, mineral, high TPC, TFC, and antioxidant contents of the fruits can be supported by some reported studies such as by Shahat et al. (2001) and Marmouzi et al. (2019) for the whole plant parts and Wang et al. (2018) and Marmouzi et al. (2019) for the fruits. The fruits also showed unique flavor and high nutritional value, high mineral contents, and high antioxidant capacity (Wang et al. 2016; Shi et al. 2018; El Maaiden et al. 2020). High amounts of major bioactive compounds such as flavonoids, alkaloids, saponins, and terpenoids, for instance, contents can be from 297 to 4078 mg/100 g (Abdoul-azize 2016). That is why there were maximum chemical contents, antioxidant potentials, and elemental (mineral) contents recorded in these four studied fruits. The present study had shown that the fruit species of *Ziziphus* (*Z. spina-christi* and *Z. jujube* fruit species) are sources of important phytochemicals, antioxidants, and minerals. The high mineral contents and antioxidant activities in the fruits suggest that they can be a good source of minerals and bioactive compounds and can be used as functional, health-enhancing foodstuffs, particularly for the prevention of diseases as supported by a previous study on Chinese *jujuba/ jujube* (Chen et al. 2017). Further characterizations of the fruits, food product developments, and the quantifications of individual phenolic, flavonoids, organic acids, anthocyanins, pectin content, and the other components fractionation and further identification/analysis should be performed.

Principal Component Analysis

Principal component analysis (PCA) is one of the most commonly used chemometric methods of multivariate analysis technique for the discriminations and differentiation of groups of samples or species (Nowicka et al. 2019). The PCA was

Table 3 EC50 radical scavenging activities of fruit crude extracts by DPPH, ABTS, and FRAP assay (3 replicates and 9 points)

Fruit	EC50 values (mg/mL) for		
	DPPH	ABTS	FRAP
Umran	3.09 ^a	5.71 ^a	11.89 ^a
Gola	3.56 ^b	7.75 ^c	15.48 ^b
Mundia	2.94 ^c	3.26 ^a	13.28 ^c
Kunkura	3.68 ^d	6.07 ^b	15.33 ^d

The post hoc descriptive multiple comparisons of DPPH, ABTS, and FRAP assays at EC50 values (mM TEAC) are based on the % radical scavenging activities of multiple point graphs (9 points) of regression equation as increase concentration (Fig. 2). Letters with a different superscript in the same column are significantly different at $p < 0.05$

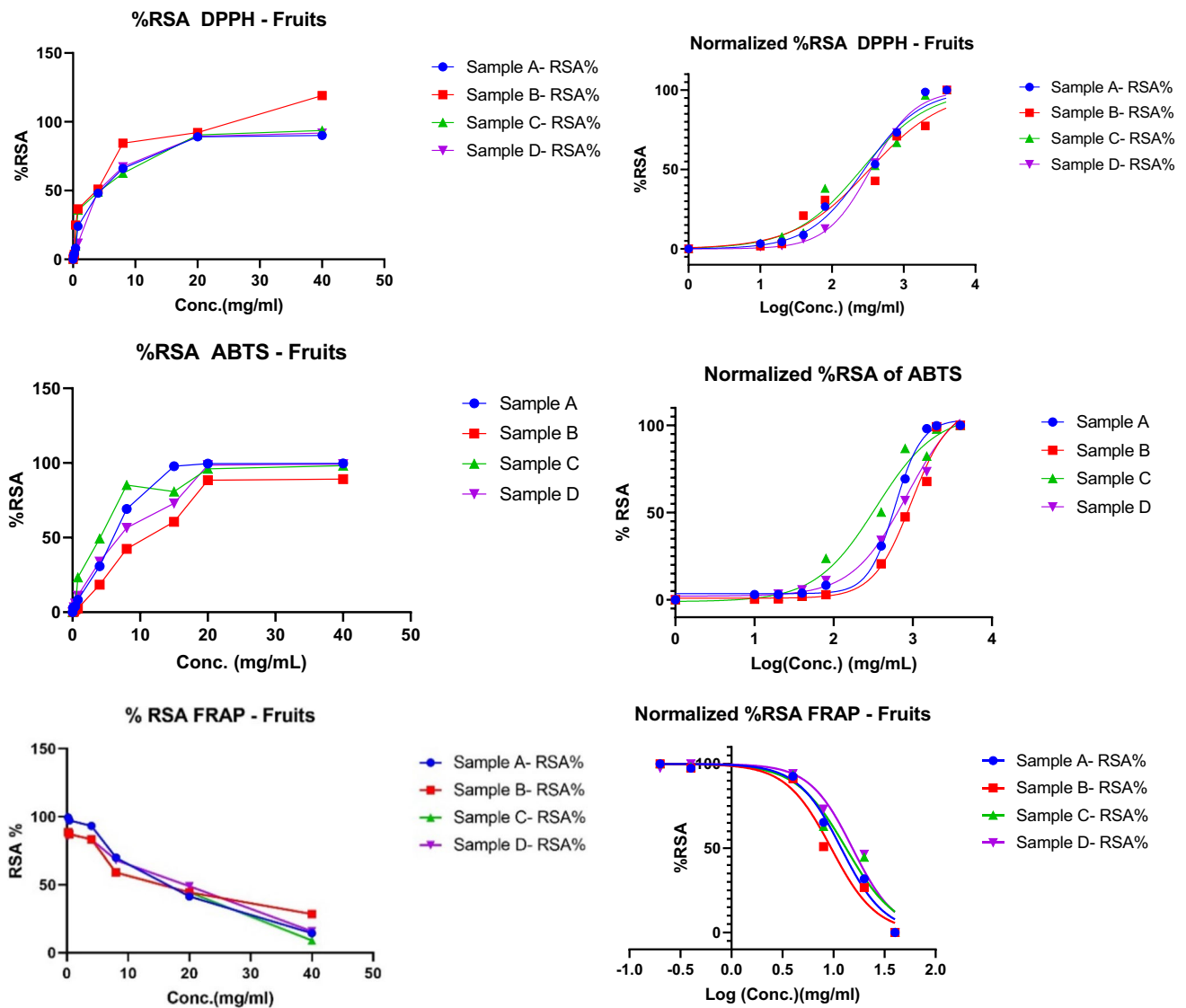


Fig. 2 Comparison of antioxidant activities of different concentrations of methanolic extract of fruit species by spectrophotometric detection of the DPPH, ABTS, and FRAP with increase concentration at 517, 734, and 593 nm, respectively

run for the minerals and antioxidants of four *Ziziphus* fruit species to look at the main data structures and association or variances among Umaran, Gola, Mundia, and Kunkura plant species, and the relationship between data and samples. In this study, PCA was run for the data obtained from fruits with different geographical origins after quantitative analysis of minerals using ICP-OES and antioxidants using a UV–VIS

spectrophotometer. The PCA was performed using Virmax with Kaiser normalization–rotation and rotated component matrix methods with eigenvalues > 1 (KMO) (Table 4, Fig. 3).

Two PCAs’ results divided the analyzed fruits into three distinct clusters (PC1, PC2, and PC3) explaining the associations and variances of minerals, antioxidants, and fruits. The first PC (PC1) of minerals explains 58.24% of the overall variations.

Table 4 Principal component analysis (PCA) results for minerals and antioxidants (total variance explained)

Minerals — total variance explained			Antioxidants — total variance explained	
Component	% of variance	Cumulative (%)	% of variance	Cumulative (%)
PC1	58.24	58.24	45.56	45.56
PC2	27.61	84.24	30.04	75.59
PC3	14.15	100.00	24.41	100.00

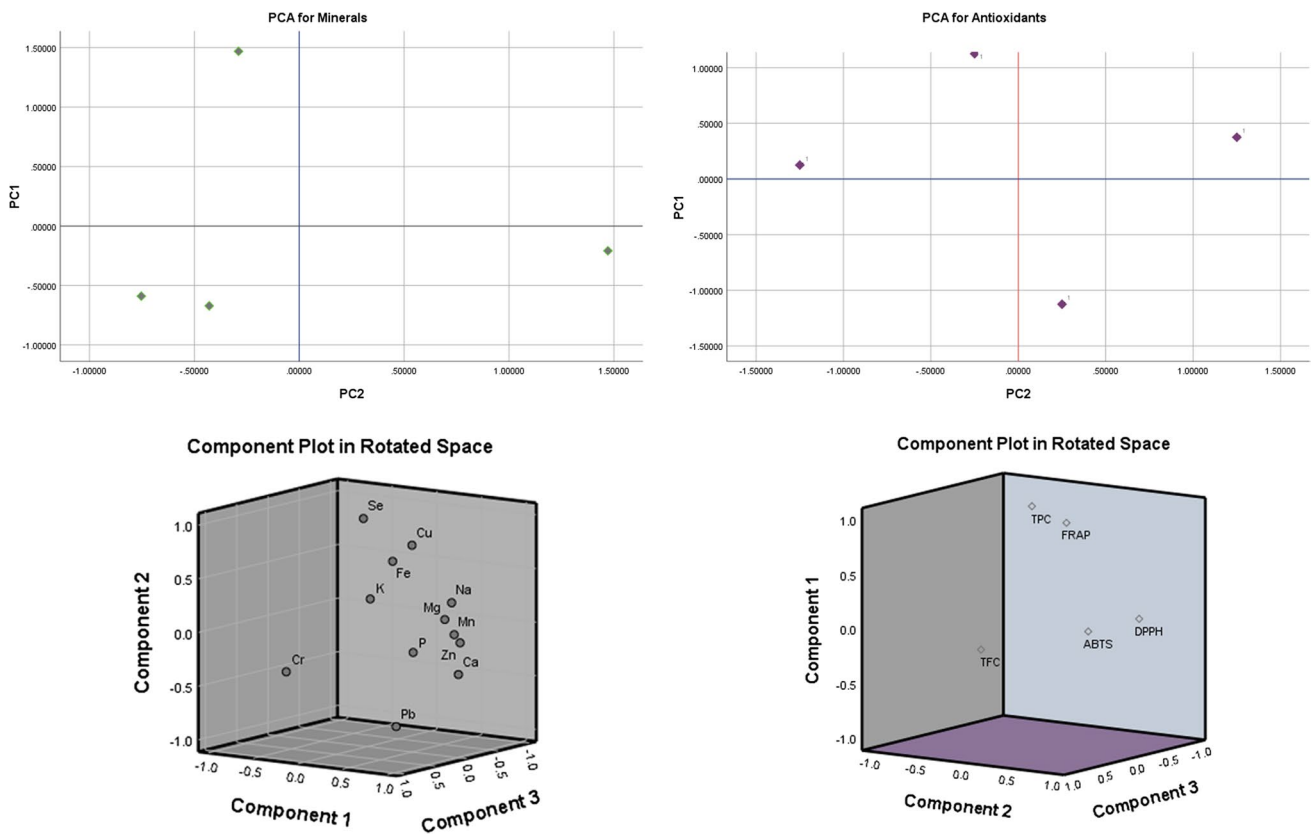


Fig. 3 Principal component analysis of minerals and antioxidants (TPC, TFC, DPPH, ABTS, and FRAP assays) for the species of *Ziziphus* (Umaran, Gola, Mundia, and Kunkura); association or vari-

The second (PC2) and the third (PC3) explain 27.61% and 14.15% variations among the studied minerals, respectively. The cumulative variance of PC1 and PC2 explained 84.24% of the overall variances of the fruits. That means the PC1 is clearly identified with Umaran (PC1), Gola and Mundia (PC2), and Kunkura (PC3) for fruit species and Na, K, Ca, Mg, P, Fe, Zn, and Pb in one cluster (PC1), Se in the second cluster (PC2), and Cr the third cluster (PC3). The factors that most contributed (strong positive association) to PC1, PC2, and PC3 (in the positive sides) were Na, Mg, Mn, Fe, P, Zn, K, Cu, and Ca (in the decreasing order), Pb, and Cr, respectively. Apart from this, on the negative side that the most contributed factor is Se, but there are no strong negative factors contributed to PC1 and PC3. Therefore, PC1 is the major contributor to the associations or variances of fruits in this study. On the other hand, the first PC (PC1) of antioxidants explains 45.56% of the overall variations. The second (PC2) and the third (PC3) explain 30.04% and 24.41% variations among the studied antioxidants, respectively. The cumulative variance of PC1 and PC2 explained 75.59% of the overall variances of the fruits. According to these analyses, the main data structure for the associations and variations of Umaran, Gola, Mundia, and Kunkura fruits is PCA of minerals. The PCA results (Fig. 3,

ation of the four plant species. Those PCAs with eigenvalues greater than one were selected for the given data, and correlations between the plant fruits were calculated using statistical package v26 between

Table 4) also showed that minerals and antioxidants have significant contributions to the strong associations of Ethiopian and Indian *Ziziphus* fruit species. Accordingly, there was a significant correlation between fruit species and their antioxidant and mineral contents. Therefore, it is clearly shown that the common feature for the Umaran, Gola, Mundia, and Kunkura fruits was the high contents of minerals and antioxidants. In addition, the PCA model indicated that the varieties of these fruits could be characterized by minerals and antioxidants and it was possible to discriminate the different *Ziziphus* species according to the geographical origin of the plants.

Conclusion

The wild edible *spina-christi* and *jujube* fruits are rich sources of various minerals, some nutrients, and antioxidant bioactive compounds. The fruits have significant antioxidant activities against various in vitro antioxidant scavenging activities. The GraphPad Prism statistical model was effective for the estimations of EC50 values for all fruits. Moreover, the studied fruit species can be used as

an easily accessible source of natural antioxidants. The consumption of these fruits may also play a vital role in fulfilling nutritional and mineral requirements and preventing various diseases, and aging. But further individual

bioactive components studies are required to confirm if these fruit species can be used as functional/nutraceutical food supplements.

Appendix

Table 5 Proximate composition of four fruit species ($n=3$, the moisture contents were estimated before drying — wet basis — but others were dry basis)

Fruit	Moisture (%)	Ash (%)	Protein (%)	Total N (%N)	Fat (%)	Carbohydrate (%)	Dry matter (%)
Umran	88.22 ± 0.10 ^a	1.92 ± 0.23 ^a	0.55 ± 0.04 ^a	0.09 ± 0.01 ^a	1.32 ± 0.04 ^a	7.99 ± 0.20 ^a	11.78 ± 0.10 ^a
Gola	83.77 ± 5.77 ^a	2.52 ± 0.08 ^a	1.07 ± 0.04 ^b	0.17 ± 0.01 ^b	8.84 ± 0.16 ^b	6.47 ± 3.63 ^a	18.89 ± 3.60 ^a
Mundia	81.77 ± 5.74 ^a	2.26 ± 0.72 ^a	1.15 ± 0.01 ^b	0.18 ± 0.00 ^b	4.98 ± 0.04 ^c	9.83 ± 6.48 ^a	18.22 ± 5.74 ^a
Kunkura	83.31 ± 9.28 ^a	3.88 ± 0.44 ^b	1.32 ± 0.04 ^b	0.21 ± 0.01 ^b	0.81 ± 0.02 ^d	10.65 ± 1.96 ^a	16.67 ± 1.53 ^a

Letters with a different superscript in the same row are significantly different ($p \leq 0.05$)

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Data Availability The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval This research did not require ethical approval.

Consent to Participate Informed consent not applicable.

Conflict of Interest Tilahun Belayneh declares that he has no conflict of interest. Sosina Gebremichael declares that she has no conflict of interest. Ajay Chinchkar declares that he has no conflict of interest. Tarekegn Berhanu declares that he has no conflict of interest. Anurag Singh declares that he has no conflict of interest. Ashutosh Upadhyay declares that he has no conflict of interest.

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