



## Research article

Bioactive composition, free radical scavenging and fatty acid profile of *Ximenia americana* grown in EthiopiaAsfawosen Mamo Bazezew<sup>a,b,\*</sup>, Shimelis Admassu Emire<sup>b</sup>, Mulugeta Teamir Sisay<sup>c</sup><sup>a</sup> Chemical Engineering Department, Adama Science and Technology University, Adama, Ethiopia<sup>b</sup> School of Chemical and Bioengineering, Department of Food Engineering, Addis Ababa Institute of Technology, P.O.B: 1176, Addis Ababa, Ethiopia<sup>c</sup> Ethiopian Institute of Agricultural Research, Ethiopia

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## ABSTRACT

*Ximenia americana* is among wild edible fruit indigenous to Ethiopia. The fruit reported as indispensable source of phytochemicals and health imparting components. However, the phytochemicals constituents, antioxidant activities and fatty acid profile of Ethiopian *X. americana* cultivar studies are still scarce. This research aimed at evaluation of flesh and seed of Ethiopian *X. americana* yellow and red colored cultivars for bioactive component, antioxidant activities, fatty acid profiles and physicochemical characteristics. The red fruit flesh had higher total phenol (3292.60 mg GAE/100 g) while yellow fruit flesh showed higher total flavonoid (173.95 mg quercetin/100g). The higher DPPH\* radical scavenging activity (97%) was observed in red fruit flesh due to its higher phenolic content. The yellow *X. americana* seed (90.69 mg QE/100g) had higher total flavonoid contents than the red seed (18.64 mg QE/100g). The dominant unsaturated fatty acid found in red *X. americana* flesh was oleic acid (26.29%), and the main saturated fatty acid detected was palmitic acid (29.78%). The seed also had high unsaturated fatty acid which was elaidic acid, (84.32%). Ximeninic acid (12.78%) which is expected to be detected in *Ximenia americana* seed oil was also observed. The highest content of fat (52.23%) and protein (16.59%) was obtained in yellow fruit seed and the lowest fat content observed in yellow flesh (7.25%). Most abundant minerals were calcium (42.45–49.55 mg/100g) and manganese (26.73–33.92 mg/100g) in seeds. Whereas, flesh displayed higher magnesium (76.3–98.27 mg/100g) and copper (1.35–1.52 mg/100g) contents. The Ethiopian *X. americana* fruit cultivars represent high nutritional and medicinal values despite variation related to genetic variability.

## 1. Introduction

Wild edible fruits can be considered as interesting high-value sources of nutrients as dietary fiber and bioactive compounds with high antioxidant activity, which could provide the basis for nutraceuticals, food supplements or functional foods (Heinrich et al., 2006). These wild edible fruits can be used as food source for the native people (Hegazy et al., 2019). Deficiencies of essential micronutrients can increase the risk of illness or death from infectious diseases by reducing immune and non-immune defenses. Such nutrient deficiencies are widespread in low and middle-income countries where wild fruits are a source of these compounds (Fernández-Ruiz et al., 2017). Scholars suggest that the consumption of wild fruit helps for healing potential of numerous diseases such as diabetes, cardiovascular problems, digestive and urinary

tract disorder as a result of their phytochemical content with diverse bioactivities Erşan et al. (2020); Islary et al. (2016).

*Ximenia americana* is one of the wild edible fruit which belongs to Olacaceae family. The fruit can be used for innovative drug formulation due to its medicinal value. Generally, the fruit is obtained in Africa, India, New Zealand, Central America and South America (Mohamed and Feyissa, 2020).

Ethiopia is known for *X. americana* fruit potential. The fruit is found in different parts of the country in which the southern region takes the upper hand with high dense quantity (Hunde, 2012). *Inkoy* is commonly known local name for the fruit. It is green colored at the early stage of ripening and turn to yellow or red colored when ripen (Maikai et al., 2009). The fruit is found in shrub form with spine. Both the yellow and red cultivars of the *X. americana* fruit had been identified in the region. The harvesting time and frequency varies from place to place depending on the availability of

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matured edible fruit due to ecological and climatic conditions (Zewdu Yilma, Ethiopian Forest and Environment Research Institute). It was observed that the red cultivar is bitter than the yellow one.

The yellow colored fruits of *Ximenia americana* grown in different countries was extensively analyzed for their nutritional, anti-nutritional composition, phytochemicals and medicinal values (Almeida et al., 2016; Lamien-Meda et al., 2008; Muhammad et al., 2019). These aforementioned reports showed that *X. americana* fruit flesh possesses considerable amount of total polyphenol, vitamin C and free radical scavenging activity. Not only the fruit flesh but also the seed presents high polyphenols and antioxidant activity which makes it potential raw material for medicinal use (Sarmiento et al., 2015). Unlike other countries, the flesh and seed of the yellow cultivar of Ethiopian *X. americana* fruit has not been studied for its phytochemicals, bioactive components and fatty acid profiles yet. Besides, as far as our knowledge is concerned, the red cultivar of *X. americana* was not studied elsewhere.

Therefore, the objective of this study was to evaluate the bio-active composition, free radical scavenging activity, fatty acid profile and physicochemical characteristics of red and yellow *X. americana* fruit fleshs and their respective seeds.

## 2. Material and methods

All chemicals and solvents used in this study were analytical grades. Methods used for the analyses were officially approved.

### 2.1. Sample collection

Red and yellow *X. americana* fruit cultivars were collected from Arbaminch area, Ethiopia. It is located at 5°58'57.04" N and 37°32'20.4" E, an altitude of 1269 m above sea level. Fruits were randomly collected from the shrubs at pick harvesting time and stored at 4 °C.

### 2.2. Sample preparation

The whole fruit flesh (includes exocarp and mesocarp) and seed of *X. americana* fruit cultivars were separated with the aid of stainless-steel knives. The flesh was homogenized (IKA, Germany) and dried using oven (FW 100, China) until constant weight was obtained. The seed sample was ground using mortar and pestle and dried using oven (FW 100, China) until constant weight was obtained. Finally, the samples were stored in cool and dry place until further analysis.

For phytochemical analysis, the flesh and seed of each cultivar samples were freeze dried at -50 °C and 0.05 mbar for 72 h using freeze dryer (Grenco N.V. S-Hertogenbosch, Holland). The samples were then ground using mortar and pestle and allowed to pass through a sieve (20 meshes). Then, it kept in a sealed polyethylene bag and stored in the dark place at ambient temperature until further analysis.

### 2.3. Phytochemical determination

#### 2.3.1. Extraction of bio-active compounds

The fruit fleshs and seeds extract were prepared using the maceration extraction according to Vongsak et al. (2013) method. The freeze dried flesh and seed powder (2 g) each was placed in 200 ml flask. Then, 40 mL of 70% Ethanol was added in to the flask. The sample was kept in a sealed flask and covered with aluminum foil to avoid light exposure. Subsequently, it was placed in the shaker (ES-20) at 100 rpm and at a temperature of 30 °C for 24 h. Whatman No.1 filter paper was used to filter the extract and dried using rotary evaporator (HS-2005S). The sample was kept in a refrigerator at 4 °C until analysis.

#### 2.3.2. Total phenolic compound

The total phenolic compound (TPC) was determined according to Dadi et al. (2018). A mixture of 0.5 mL of the extract and 2.5 mL of the 10 % aqueous (by volume) Folin-Ciocalteu reagent was prepared. 8 min

later, 2.0 mL of the 7.5 % (*m/v*) sodium carbonate was added. Then, it mixed with the extract mixture and kept in the dark at room temperature for 2 h. Similar method was used for the blank and gallic acid standard prepared at different concentrations (0, 20, 40, 60, 80, 100, 120, 140, 160 and 180 µg/mL) to develop a standard curve. UV-Vis spectrophotometer (PerkinElmer, Lambda 950, UK) was used to measure the absorbance at 765 nm. The result was stated in terms of mg gallic acid equivalent per 100 g (mg GAE/100 g).

#### 2.3.3. Total flavonoid content

The total flavonoid content (TFC) was analyzed following Adom and Liu (2002) method. First the extract was mixed with 0.15 mL of 5% (*m/v*) sodium nitrite. Then, 2.5 mL of de-ionized water was used and mixed. The mixture was kept for 6 min. 10 percent (*m/v*) aluminum chloride (0.3 mL) was added and mixed. This was followed by addition of 1 mL of 1.0 M sodium hydroxide and subsequently 0.55 mL of distilled water. The solution was kept for 15 min in stirring. UV-Vis spectrophotometer (PerkinElmer, Lambda 950, UK) was used for reading of the concentration at 510 nm. The same procedure was applied for the blank and quercetin as standard at different concentrations (25, 50, 75, 100, 125, and 150 µg/mL) to develop the standard curve. The TFC was expressed as mg quercetin equivalent per 100 g of dry mass (mg QE/100g) of the sample.

### 2.4. DPPH\* free radical scavenging

It was quantified using the 2,2-diphenyl-1-picrylhydrazyl (DPPH\*) according to Brand-Williams et al. (1995). A mixture of 2.4 mg of DPPH\* solution in 100 mL of 80 % ethanol was prepared. The absorbance was ensured for the reading of less than one at 517 nm. One tenth milliliter of the sample and standard (ascorbic acid) of different concentrations was mixed with 3.9 mL of DPPH\* solution. The mixtures were kept in the dark for 30 min. Absorbance reading was taken using UV-Vis spectrophotometer (PerkinElmer, Lambda 950, UK) at 517 nm. The solution for the blank was prepared using 80% methanol instead of the sample solution and assayed under the same conditions. The absorbance was then read. The DPPH\* scavenging capacity was calculated against blank (Eq.1). The concentration providing 50% scavenging of DPPH\* free radicals (IC<sub>50</sub>) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration versus the corresponding scavenging effect.

$$\text{DPPH}^* \text{ scavenging (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \quad (1)$$

where:  $A_{\text{blank}}$  is control sample absorbance.

$A_{\text{sample}}$  is test sample absorbance.

### 2.5. Fourier transform infrared spectroscopy

The Fourier Transform Infrared Spectroscopy (FTIR) analysis was done according to Park et al. (2015). A Perkin Elmer IR SUBTECH Spectrum ASCII PEDS spectrophotometer from 4000 to 400 cm<sup>-1</sup> range, by Fourier Transformed and KBr pellets was used to obtain the absorption spectra in infrared region for the yellow and red *X. americana* fruit flesh and seed.

### 2.6. Fatty acid profile

Fatty Acid Methyl Esters (FAMES) were prepared by trans- esterification of the red *X. americana* flesh and its seed oil obtained after Soxhlet extraction according to David et al. (2005). About 100-mg sample was weighed in a 20-mL test tube. The sample was mixed in a 10-mL hexane. 100-µL 2 N potassium hydroxide in methanol (11.2 g in 100 mL) was added. The test tube was vortexed for 30 s and centrifuged. The clear

supernatant was transferred in to 2-mL auto sampler vial. The results were presented in relative percentage of each fatty acid.

The quantification of fatty acids was performed using automated gas chromatograph (Agilent 6890 GC) with a flame ionization detector (FID). Automated split-splitless injector (Agilent 7683) was used for sample injection. The separation was conducted on a 60 m × 0.25 mm ID, 0.15 µm columns. The inlet and detector temperatures were 250 °C and 280 °C respectively. Helium was used as carrier gas at the pressure of 230 Kpa. The injection volume was 1 µL and the Split ratio was 1/50.

## 2.7. Chemical compositions

### 2.7.1. Moisture content

Moisture content was determined gravimetrically according to an AOAC method No. 925.09 (2000) method with some modifications immediately after harvesting on fresh weight basis.  $5 \pm 0.05$  g flesh and seed samples from both *X. americana* were dried in a vacuum oven (Model 4567, Kimya Pars Co., Iran) at 65 °C until a constant weight was obtained. The percent moisture content was computed by weight difference after oven drying to that of the original sample weight.

### 2.7.2. Crude fat

Crude fat content was determined according to AOAC method No. 925.38 (2000). Soxhlet extractor (Soxtec™ 8000) was used following the procedure on the manufacturer user guide manual.  $2.0 \pm 0.05$  g of partially oven dried flesh and seed samples from both *X. americana* fruit cultivars were weighed in a filter bag and sealed. The fat was extracted by refluxing the filter bags containing the samples for 60 min in a petroleum ether. After extraction, filter bags were dried in the oven at  $102 \pm 2$  °C for 15 min and the crude fat content was computed by the weight difference to that of the initial sample weight.

### 2.7.3. Crude fiber

The crude fiber was determined according to AOAC method No. 962.09 (2000) standard methods using auto-fiber analyzer (Fibertec™ 8000).  $2 \pm 0.05$  g of oven dried flesh and seed samples were defatted using 250 ml petroleum ether. The sample digestion for crude fiber was carried out using 1N of sulfuric acid and potassium hydroxide, according to manufacturer's instructions. After digestion, bags were rinsed with boiling water followed with acetone and air dried. The crude fiber was calculated based on the weight difference after digestion to that of the initial sample weight.

### 2.7.4. Crude protein content

The total nitrogen content of sample was determined using Kjeldahl method AOAC No. 920.87 (2000).  $0.5 \pm 0.05$  g of flesh and seed samples of both fruits were used for crude protein analysis. Crude protein content was calculated by multiplying the total nitrogen with a factor of 6.25.

### 2.7.5. Determination of ash and mineral composition

Ash and mineral compositions had been determined as described by Bouhlali et al. (2017). Briefly, about 2 g of each *X. americana* fruit samples were placed in a previously weighed porcelain crucible and heated at 550 °C in a furnace for 4 h until the residue was uniformly white. The resulting ashes were dissolved in 5 mL of concentrated hydrochloric acid and the mixtures were heated on a hot plate until complete dryness. Then, little drops of H<sub>2</sub>O and 5 mL of distilled water were added and made up to 25 mL in a standardized flask. The resultant solutions have been used for mineral content analysis. An atomic absorption spectrometry (Agilent AAS 240 series, USA, 2017) was used to determine Ca, Cu, Fe, Mg, and Mn contents.

### 2.7.6. pH and total soluble solid determination

pH was determined as described by (Holcroft and Kader, 1999) method with some modifications. About 10 g of sample was diluted with 100 mL of deionized water and analyzed using a pH meter (LE-409,

Germany) with automatic temperature adjustment. Digital refractometer (RFM C-60, Germany) was used to examine the total soluble solid content.

## 2.8. Statistical analysis

All experiments were conducted in triplicate and values were reported as mean ± standard deviation. JMP software (Version 13.0, SAS institute Inc.) was used to analyze the data. The mean values ( $P < 0.05$ ) for the data was compared using one way analysis of variance (ANOVA) and the Tukey's test.

## 3. Result and discussion

### 3.1. Phytochemical components

The total phenolic and flavonoid content of the yellow and red colored *X. americana* fruit cultivars were depicted in Table 1. Both fruits' seeds and flesh s of *X. americana* showed significant ( $P < 0.05$ ) differences in total phenol and total flavonoid compound.

#### 3.1.1. Total phenolic compound

The total phenolic content showed significant difference ( $P < 0.05$ ) between the flesh of the red (3292.60 mg GAE/100 g) and yellow *X. americana* fruit (2880.39 mg GAE/100 g). This variation perhaps explained by the taste of both fruits in which the red colored fruit flesh tasted bitter than the yellow one. Almeida et al. (2016) and Sarmiento (2015) reported that the total phenolic content of the yellow *X. americana* fruit grown in different region of Brazil were 1298.22 mg GAE/100 g and 3002.08 mgGAE/100 g, respectively. Lamien-Meda et al. (2008) reported lower total phenol content of *X. americana* fruit (2086.67 mg GAE/100 g) grown in Burkina Faso as compared to the present study.

The red and yellow *X. americana* seeds contained 704.31 mg GAE/100 g and 544.04 mg GAE/100 g of total phenolic content, respectively. Differences ( $P < 0.05$ ) were observed in total phenolic content within the seeds and fleshs of the red and yellow *X. americana* fruits, with the fleshs contained higher mean values (Supplementary material, Table 1). The total phenolic content of the red fruit might contain anthocyanin pigment at low pH (2.84 in this case) which was responsible for its reddish color. pH is an important factor for color expression of anthocyanin, being these compounds more stable in acidic than alkaline or neutral medium (de Freitas et al., 2017).

Folin-Ciocalteu method used for determination of polyphenol may have some errors in increasing the yield due to reactivity of the reagent with other compounds like vitamin C (Everette et al., 2010). However, Almeida et al. (2016) observed this effect for the yellow *X. americana* fruit and concluded that there was no vitamin C interference in polyphenol yield. The author reported that no change was seen in vitamin C during maturing stages as the polyphenol decreased by half.

The difference in total phenolic content between the two fruits could be explained by their genetic factors and different ability to synthesize secondary metabolites. The substantial content of phenolic compound of the red colored *X. americana* fruit can be an important raw material for functional foods, food additives and pharmaceuticals.

#### 3.1.2. Total flavonoid content

The total flavonoid content of the yellow *X. americana* fruit flesh (173.95 mg QE/100 g) was significantly higher ( $P < 0.05$ ) than the red *X. americana* flesh (58.34 mg QE/100 g). The obtained result in this study were higher than reported value of 30.95 mg QE/100 g by Lamien-Meda et al. (2008) in Burkina Faso. The total flavonoid content of yellow *X. americana* seed (90.69 mg QE/100 g) was almost four times higher than the red *X. americana* seed (18.64 mg QE/100 g), with significant difference ( $P < 0.05$ ). There was significant difference ( $P < 0.05$ ) in total flavonoid content between the two *X. americana* fruits in terms of flesh and seed which might be described due to their color variation

**Table 1.** Total phenol and flavonoid content of two cultivars of *X. americana* fruit.

Parameters	Red <i>X. americana</i>		Yellow <i>X. americana</i>	
	Seed	Flesh	Seed	Flesh
Total Phenol (mg GAE/100 g)	704.31 ± 19.84 <sup>c</sup>	3292.60 ± 259.21 <sup>a</sup>	544.04 ± 34.56 <sup>d</sup>	2880.39 ± 198.49 <sup>b</sup>
Total Flavonoid (mg QE/100 g)	18.64 ± 4.27 <sup>d</sup>	58.34 ± 6.62 <sup>c</sup>	90.69 ± 7.57 <sup>b</sup>	173.95 ± 8.24 <sup>a</sup>

The same letters in the same row represents no significant difference ( $P < 0.05$ ). All values are expressed as mean ± standard deviation ( $n = 3$ ).

(Supplementary material, Table 1). It is rational to accept as true that the yellow *X. americana* fruit is rich in yellow flavonoid content. This was confirmed by Almeida et al. (2016) who reported that the yellow flavonoid was almost twenty times higher than the total anthocyanin for the yellow *X. americana* fruit. These differences in flavonoid content might be emanated from the dependency of either on the cultivar or on the growing condition (Al-Alawi et al., 2017).

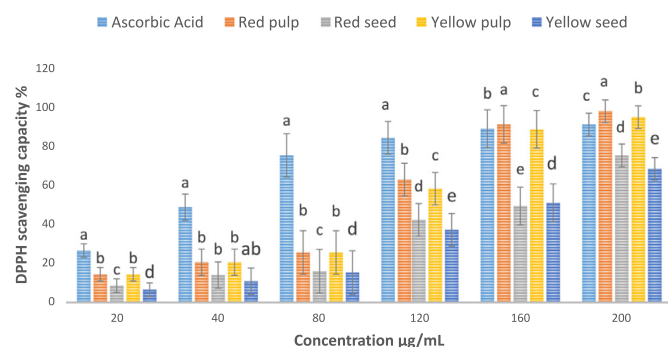
The fruit cultivars color showed variations during maturation from green to red and yellow. These variations of fruits during maturation are dependent on the concentrations of anthocyanin pigments, yellow flavonoid content along with chlorophyll and carotenoids as well as the interactions between them (Xu et al., 2016). The presence of significant quantity of total phenols and flavonoids in the fruits have role in radical scavenging activities, lowering some chronic diseases, prevention of some cardiovascular disease and certain kinds of cancer development (Metoui et al., 2019).

### 3.2. DPPH\* free radical scavenging

A stable free radical used to determine the ability of fruit extract to scavenge free radicals is known as DPPH\* (Silva and Sirasa, 2018). The scavenging of free radicals both in fruit parts in this study was dependent on the concentration of the flesh and seed extract (Figure 1). Both the red and yellow *X. americana* fruit flesh depicted more than 90% of DPPH\* free radical scavenging at a concentration of 200 µg/mL. The red flesh of *X. americana* fruit showed higher radical scavenging (97%) as compared to the yellow *X. americana* flesh (94%) at 200 µg/mL concentration. Researchers from different countries Lamien-Meda et al. (2008); Le et al. (2012) and Sarmento (2015) reported that the yellow *X. americana* fruit had high antioxidant activity which agree with the current study.

The yellow and red *X. americana* seeds also showed significant amount of DPPH\* radical scavenging at 200 µg/mL concentration. The red *X. americana* seed had higher scavenging activity (75%) than the yellow *X. americana* seed (68%). This might be due to the presence of the relative higher inherent bioactive compound like total phenol in the fruit which enables to be used in food and pharmaceuticals to prevent oxidation of cells in human body.

The quantity of antioxidant needed to decrease the original amount of free radicals by half is called IC<sub>50</sub>. The IC<sub>50</sub> for the red *X. americana* flesh was 99 µg/mL which indicated the highest antioxidant activity among all



**Figure 1.** DPPH\* scavenging capacity (%) of *X. americana* fruit parts at different concentrations of extract.

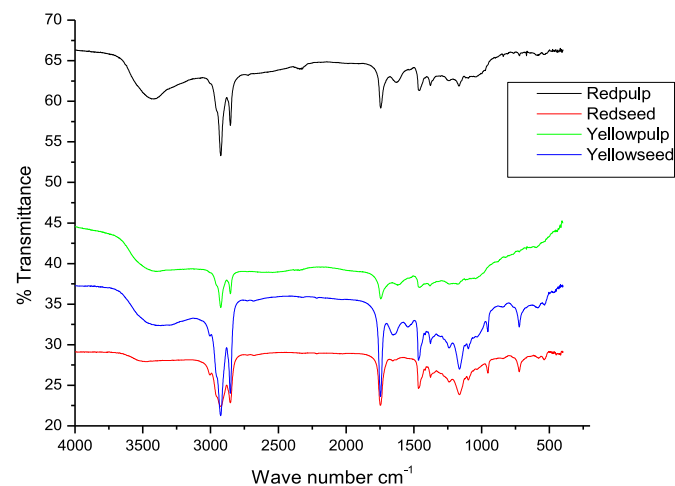
studied *X. americana* fruit parts. It was obtained that the IC<sub>50</sub> value for the yellow *X. americana* flesh was (102 µg/mL), which had no significant difference with the red *X. americana* flesh. The red *X. americana* seed also showed higher antioxidant capacity (IC<sub>50</sub> = 147 µg/mL) as compared to yellow fruit seed (IC<sub>50</sub> = 154 µg/mL) without significant variations. Sarmento (2015) reported that the flesh and seed of the yellow *X. americana* fruit grown in Mossoró-Assu, RN, Brazilian semiarid region had also significant antioxidant activities. Hence, the fruit can be applied for the value addition of food processing and pharmaceutical industry due to the high free radical scavenging activities (Schubert et al., 2007).

### 3.3. FTIR

From the FTIR spectrum, it was observed that similarities in the absorption bands with slight differences in the intensity of the peaks for the red and yellow *X. americana* fruit fleshs and seeds (Figure 2).

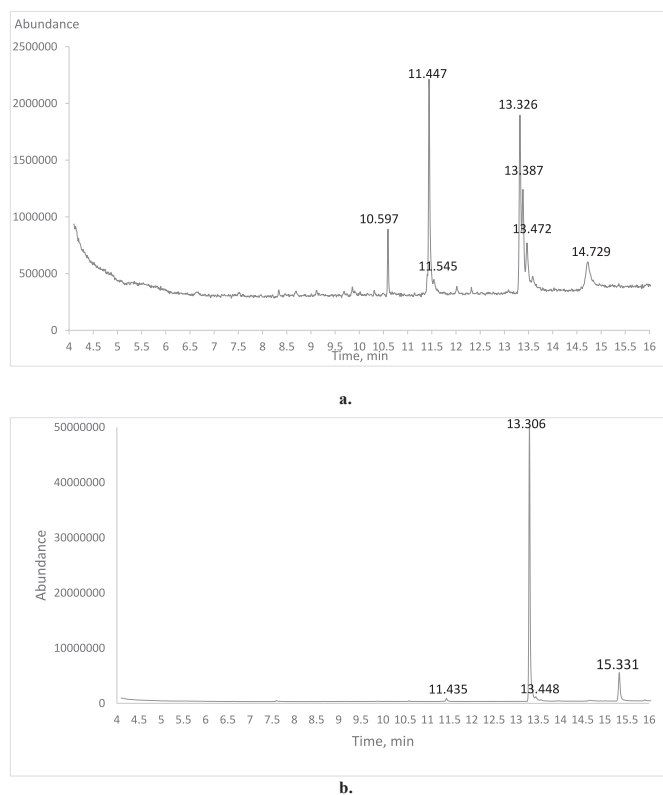
The narrow and weak peak at 2834 cm<sup>-1</sup> and 2828 cm<sup>-1</sup> wave number for the red and yellow *X. americana* fleshs, respectively represents the presence of symmetric stretching vibrations of aldehyde (-CH<sub>2</sub>) bond and methoxy groups which confirmed the presence of phenolic compounds (Stuart, 2000). The peak at 2541 cm<sup>-1</sup> and 2545 cm<sup>-1</sup> for the red and yellow *X. americana* fleshs, respectively revealed the presence of S-H stretching bond (thiols) functional group (Stuart, 2005). The strong and narrow peak at 1076 cm<sup>-1</sup> for both *X. americana* fleshs was attributed to the presence of -C-O stretching (primary alcohol) functional group (Stuart, 2000). Presence of aldehyde functional group confirmed the presence of xylose and arabinose which contribute to the sweetness of the *X. americana* fruit flesh. -C-O stretching (primary alcohol) may indicate presence of hydroxyl group in phenolic compound and sugar component.

The weak peak at 3462 cm<sup>-1</sup> for the red *X. americana* seed revealed the presence of N-H stretches (amine) functional group (Stuart, 2000). According to the red and yellow *X. americana* seeds spectrum, the vibrational frequencies found around 3278 cm<sup>-1</sup> for both seeds and 3047 cm<sup>-1</sup> for the red seed represents the C-H stretching vibration of cis



**Figure 2.** IR spectrum of the flesh and seed of the red and yellow *X. americana* fruit cultivar.





**Figure 3.** GC-MS chromatogram for the fatty acid profile of the red *X. americana* fruit cultivar; a. Flesh, b. Seed.

double bond (=CH) alkene functional groups (Stuart, 2000). The peak at  $2838\text{ cm}^{-1}$  and  $2837\text{ cm}^{-1}$  for the red and yellow *X. americana* seeds respectively refers the presence of symmetric stretching vibrations of aldehyde ( $-\text{CH}_2$ ) bond (Stuart, 2000). The peak at  $2535\text{ cm}^{-1}$  for both fruit seeds revealed the presence of S-H stretching bond (thiols) functional group (Stuart, 2000). The presence of  $-\text{C}\equiv\text{N}$  (nitrile) functional group attributed by a peak at  $2254\text{ cm}^{-1}$  on both seeds spectrum (Stuart, 2000). A peak found at  $1075\text{ cm}^{-1}$  and  $1076\text{ cm}^{-1}$  for the red and yellow *X. americana* seeds, respectively indicated the presence of  $-\text{C}-\text{O}$  stretching functional group (Stuart, 2000). Aldehyde and  $\text{C}-\text{O}$  functional groups confirmed the presence of phenolic and flavone hydroxyl group.

### 3.4. Fatty acid profile

Figure 3 shows GC-MS chromatogram for the fatty acid profile of the red *X. americana* cultivar for its flesh and seed. The dominant saturated fatty acid found in flesh was palmitic acid (29.78%), whereas the main unsaturated fatty acid detected was oleic acid, methyl ester (26.29%) (Table 2). Similarly, the main saturated fatty acid in seeds was stearic acid, methyl ester (1.72%) while the main unsaturated fatty acids were Elaidic acid, methyl ester (84.32%) and Ximeninic acid (12.78%). *X. americana* seed is one of the source for ximeninic acid that may not be found in most fruits. These results vary with the reported value of the same species by Eromosele and Eromosele (2002) and Tanko et al. (2017). This might be due to genetic variability and environmental variations.

The presence of high proportion of palmitic acid methyl ester in flesh makes it a good candidate for margarine and other fat based product manufacturing (Trabelsi et al., 2012). Oleic acid, methyl ester existence in flesh helps to maintain some situations on metabolism, cancer and cardiac diseases (Lee et al., 2012). On the other hand, the presence of high fraction of total unsaturated fatty acid makes the Ethiopian *X. americana* fruit seed a preferred input for the industrial application.

The fatty acid profile result indicated the *X. americana* seed and flesh oil to be used for essential oil production.

### 3.5. Chemical compositions

The chemical composition result showed variation between the red and yellow *X. americana* fleshs and seeds (Table 3). Statistical differences between the two fruit seeds were observed in fat, and protein content. Their flesh also showed significant differences ( $P < 0.05$ ) in ash content.

#### 3.5.1. Moisture content

Significant differences were not observed for the moisture content of the red (68.0%) and yellow (71.0%) *X. americana* fleshs. The moisture content of the flesh of both fruits in this study were in agreement with Sarmiento et al. (2015) report studied in Brazil. The study fruit fleshs had also similar moisture content as compared to berry-like fruits of *Hypericum androsaemum L* which ranged from 71.60% in ripe (black) fruits to 76.62% in unripe (red) fruits (Caprioli et al., 2016). The red and yellow *X. americana* fruit seeds were also found to be (15.62%) and (14.09%) in fresh weight basis, respectively. There were no significant variations between moisture content of both *X. americana* seeds. The relative low moisture content indicated that the seeds will have excellent keeping quality, decreases the probability of microbial growth, unwanted fermentation, premature seed germination and many undesirable biochemical changes normally associated with these processes (Kaleta and Górnicki, 2013).

#### 3.5.2. Crude protein

The yellow (11.11%) and red (10.32%) *X. americana* flesh protein content didn't show significant differences ( $P < 0.05$ ). Significant regional differences were also observed on protein content of *X. americana* fruit from South America to Africa. The study fruit fleshs showed higher protein content as compared to the reported value by Lockett (2000); Muhammad et al. (2019) and Sarmiento et al. (2015) for the yellow *X. americana*. This variation in protein content could be explained by agro-ecological variations such as climate, weather and soil type.

The yellow and red *X. americana* seeds protein content were 16.59% and 14.69% respectively, and were significantly different ( $P < 0.05$ ). Both *X. americana* seeds presented higher protein content as compared to the fleshs. This might be due to the seeds used as protein storage site for the germination and seedling growth (Shewry et al., 1995). Besides, the high protein content of the seeds of *X. americana* makes suitable for use as feed stocks for animals.

#### 3.5.3. Crude fat

The fat content for the red (7.78%) and yellow flesh (7.25%) had no significant differences. The fat content of *X. americana* fruit in this study was in agreement with the reported value by Muhammad et al. (2019) and Saeed and Bashier (2010). The result obtained from the fat analysis revealed that both fruit seeds contained higher fat content than their respective fleshs. The red seed value (49.58%) was significantly lower than the yellow fruit seed (52.23%). The seeds possessed almost seven times higher fat content than the fleshs, with significant variations between the two seeds ( $P < 0.05$ ). High fat content of the seed indicated as the fat is stored in the seed (Nwofia et al., 2012).

#### 3.5.4. Crude fiber

Significant variation was not observed in crude fiber content between the red (5.58%) and yellow (4.41%) *X. americana* fleshs. Lower crude fiber content of *X. americana* flesh (3%) was reported by Muhammad et al. (2019). The study fruit flesh had low fiber content when it is compared with similar genus *X. caffra* (Getachew et al., 2013). The *Alphonsa* variety mango fruit flesh in Ethiopia had higher crude fiber content (23.07%) as compared to the study fruit flesh (Arumugam and Manikandan, 2011). The crude fiber content of the red (28.09%) and

**Table 2.** Fatty acid composition (%) of flesh and seed of the red *X. americana* fruit cultivar.

Retention time (min)	Systematic name	Common name	Chemical formula	Flesh	Seed
10.59	Nonadecane	Nonadecane	C <sub>19</sub> H <sub>40</sub>	6.36	ND
11.44	Hexadecanoic acid, methyl ester	Palmitic acid methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	29.78	1.18
11.54	2-Chloro-N-(hydroxymethyl)acetamide	Chloracetamide-N-methanol	C <sub>3</sub> H <sub>6</sub> ClNO <sub>2</sub>	1.77	ND
13.32	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	26.29	ND
13.30	9-Octadecenoic acid, methyl ester (E)	Elaidic acid, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	ND	84.32
13.38	Octadecane, 2,6,10,14-tetramethyl	2,6,10,14-Tetramethyloctadecane	C <sub>22</sub> H <sub>46</sub>	16.29	ND
13.47	Methyl stearate	stearic acid, methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	8.18	1.72
14.72	Bis(2-ethylhexyl) phthalate	Diethylhexylphthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	11.33	ND
15.33	Methyl octadeca-9-yn-11-trans-enoic acid	Ximeninic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	ND	12.78
TSFA	-	-	-	73.71	2.9
TUFA	-	-	-	26.29	97.1

ND - Not detected. TSFA - Total saturated fatty acid; TUFA- Total unsaturated fatty acids.

yellow (25.22%) *X. americana* seeds were not statistically different. The present study indicated that fiber from *X. americana* seeds can be a potential source for functional food development. The crude fiber availability in the fruits promotes treating diabetics commencement (Hassan et al., 2008).

### 3.5.5. Total ash

The ash content of the fruits could be as an index of mineral contents. There were statistical differences ( $P < 0.05$ ) in ash content between both fruits which resulted 7.83% for the red and 7.02% for the yellow *X. americana* flesh. These variations might be described by environmental factors such as climate, weather and soil type (Maathuis and Diatloff, 2013). It was observed that the present study had higher ash content as compared to previous study of *X. americana* fruit flesh by Sarmiento et al. (2015). However, the study fruit had lower values of ash content with the reported value of Muhammad et al. (2019). The ash content of the red and yellow *X. americana* seeds were (1.45 %) and (1.32%) respectively. Both fruit seeds didn't show significant differences statistically. Sarmiento et al. (2015) reported similar values for the ash content of *X. americana* seed.

### 3.5.6. pH and total soluble solids

The pH of the fruit appears to indicate its desirability in food processing industry in relation with imparting different flavor and odor (Silva et al., 2013). There was significant difference ( $P < 0.05$ ) between the pH of the red (2.84) and the yellow (2.75) *X. americana* fruit flesh. In contrast, no statistical variation was observed between the red (4.91) and yellow (4.79) *X. americana* seeds. The acidity of the flesh were twice higher than their respective seeds and revealed the acidic nature of the fruit (Table 3). This acidic nature of the fruit was probably due to the oxidation of pyruvic acid in kreb's cycle which results high amount of

organic acids in fruits (Silva et al., 2013). Previous studies reported similar pH value of yellow *X. americana* fruit Almeida et al. (2016); da Silva et al. (2008) and Sarmiento et al. (2015).

The total soluble solid (TSS) showed no significant variation in which 26.82 °Bx was for the red and 25.57 °Bx was for the yellow *X. americana* flesh. We observed that the natural flavor and taste of the fruit was correlated with total soluble solid content. TSS taken as quality indicator in sweetness of fresh and processed horticultural products (Magwaza and Opara, 2015). Besides, de Oliveira et al. (2014) reported that the higher TSS plays a great role in minimizing the energy cost for food processing required for removal of retained water. The present study showed higher TSS as compared to similar fruit (Almeida et al., 2016); (da Silva et al., 2008); (Mora et al., 2009) and (Sarmiento et al., 2015).

### 3.6. Mineral content

Minerals are vital for the maintenance of human health. The mineral content in this study showed variations between the yellow and red *X. americana* fruit cultivars (Supplementary material, Table 4). The seeds contained significantly higher amount of calcium and manganese as compared to the respective flesh.

The calcium content of the red *X. americana* flesh (16.56 mg/100g) was significantly different ( $P < 0.05$ ) from the yellow one (23.14 mg/100g). The red *X. americana* seed (49.55 mg/100g) was significantly different ( $P < 0.05$ ) with the yellow *X. americana* seed (42.45 mg/100g) in calcium content. Lockett (2000) and Sarmiento et al. (2015) reported lower calcium content of the yellow *X. americana* fruit as compared to this study. The Ethiopian *X. americana* fruit promotes the development of bones and teeth as well as for a number of metabolic functions in the body due to its considerable content of calcium.

**Table 3.** Chemical composition of red and yellow *X. americana* fruit cultivar expressed in dry weight basis except moisture.

Chemical compositions	Red <i>X. americana</i>		Yellow <i>X. americana</i>	
	Seed	Flesh	Seed	Flesh
Moisture (% fw)	15.62 ± 0.60 <sup>b</sup>	68 ± 0.59 <sup>a</sup>	14.09 ± 0.49 <sup>b</sup>	71 ± 0.70 <sup>a</sup>
Crude fat (%)	49.58 ± 0.37 <sup>b</sup>	7.78 ± 0.31 <sup>c</sup>	52.23 ± 0.43 <sup>a</sup>	7.25 ± 0.33 <sup>c</sup>
Total Nitrogen	2.39 ± 0.31 <sup>b</sup>	1.65 ± 0.25 <sup>c</sup>	2.65 ± 0.41 <sup>a</sup>	1.77 ± 0.23 <sup>c</sup>
Crude protein (N × 6.25)	14.96 ± 1.34 <sup>b</sup>	10.32 ± 1.35 <sup>c</sup>	16.59 ± 2.25 <sup>a</sup>	11.11 ± 1.52 <sup>c</sup>
Crude fiber (%)	28.09 ± 1.34 <sup>a</sup>	5.58 ± 0.51 <sup>b</sup>	25.22 ± 1.93 <sup>a</sup>	4.41 ± 0.80 <sup>b</sup>
Ash (%)	1.45 ± 0.23 <sup>c</sup>	7.83 ± 0.25 <sup>a</sup>	1.32 ± 0.22 <sup>c</sup>	7.02 ± 0.22 <sup>b</sup>
pH	4.91 ± 0.1 <sup>a</sup>	2.84 ± 0.01 <sup>b</sup>	4.79 ± 0.09 <sup>a</sup>	2.75 ± 0.01 <sup>c</sup>
Total soluble solid	ND	26.82 ± 3.01 <sup>a</sup>	ND	25.57 ± 1.51 <sup>a</sup>

The same letters in the same row represents no significant difference ( $P < 0.05$ ). All values are expressed as mean ± standard deviation (n = 3). fw - fresh weight. ND: Not determined.

**Table 4.** Mineral composition of seed and flesh of *X. americana* fruit (expressed on mg/100 g) of Sample.

Fruit parts	Calcium	Magnesium	Iron	Manganese	Copper
Red flesh	16.56 ± 2.08 <sup>d</sup>	98.27 ± 11.02 <sup>a</sup>	2.16 ± 13 <sup>a</sup>	0.74 ± 09 <sup>c</sup>	1.35 ± 04 <sup>ab</sup>
Red seed	49.55 ± 6.9 <sup>a</sup>	32.64 ± 4.0 <sup>d</sup>	1.15 ± 0.05 <sup>b</sup>	33.92 ± 0.58 <sup>a</sup>	0.083 ± 0.09 <sup>c</sup>
Yellow flesh	23.14 ± 1.8 <sup>c</sup>	76.3 ± 5.19 <sup>b</sup>	1.74 ± 0.8 <sup>a</sup>	1.07 ± 0.35 <sup>c</sup>	1.52 ± 0.02 <sup>a</sup>
Yellow seed	42.45 ± 14 <sup>b</sup>	41 ± 288 <sup>c</sup>	1.29 ± 0.01 <sup>b</sup>	26.73 ± 7.08 <sup>b</sup>	0.10 ± 0.5 <sup>bc</sup>

Means followed by the same letters in the same column for each part of the fruit do not differ by Tukey's test at 5% probability. All values are expressed as mean ± standard deviation (n = 3).

The red *X. americana* flesh showed the higher concentration in magnesium content (98.27mg/100g) as compared to the yellow flesh (76.3 mg/100g). Muhammad et al. (2019) and Sarmiento et al. (2015) observed lower amount of magnesium content in yellow *X. americana* flesh. On the other hand, Lockett (2000) reported the highest value of magnesium in yellow *X. americana* flesh, 145 mg/100g. Significant variation in magnesium content ( $P < 0.05$ ) was observed in seeds and fleshs of the yellow and red *X. americana* fruits. The higher magnesium content in *X. americana* flesh helps in making cofactor for different crucial enzyme systems, it's needed for DNA and RNA synthesis increment and energy generation for mitochondria to carry out oxidative phosphorylation (Hegazy et al., 2019).

The iron content of yellow (1.74 mg/100g) and red (2.16 mg/100g) *X. americana* fleshs didn't show significant differences. No significant variations were also observed for the red (1.15 mg/100g) and yellow (1.29 mg/100g) *X. americana* fruit seeds. These values were higher than the reported value by Sarmiento et al. (2015) and lower than Lockett (2000) report for the yellow *X. americana*. Consuming such fruit will help in maintaining a healthy immune system and disease prevention and aiding in energy production due to its higher iron content (Beard and Dawson, 1997).

The red and yellow *X. americana* fleshs didn't show significant variations in manganese content (0.74 mg/100g) and (1.07 mg/100g), respectively. However, the red *X. americana* seed (33.92mg/100g) was significantly higher ( $P < 0.05$ ) than the yellow seed (26.73 mg/100g) in manganese content. The result revealed that the study fruits had higher manganese content in seed and lower values in its flesh. Sarmiento et al. (2015) reported lower values of manganese content than the present study. Sereno et al. (2018) reported lower manganese content (0.04 mg/100 g) of *Solanum sessiliflorum* Dunal wild fruit which had lower values as compared to the study fruits.

The copper content of the red (1.35 mg/100g) and yellow (1.52 mg/100g) *X. americana* fleshs were significantly different ( $P < 0.05$ ). On the other hand, the red seed (0.083 mg/100g) had lower copper content than the yellow (0.10 mg/100g) *X. americana* fruit seed. Lockett (2000) and Sarmiento et al. (2015) reported lower copper content in flesh and seed of yellow *X. americana* fruit as compared to the current study.

The *X. americana* fruit parts investigated in the present study showed variation in their mineral content. The fruits might be considered as a good source for minerals which could be vital to improve the human body immune system and helps in fighting many diseases.

#### 4. Conclusion

The results of the present study illustrate characterization of the red and yellow *X. americana* fruit grown in Ethiopia. The yellow *X. americana* fruit had higher flavonoid content while the red *X. americana* fruit contained higher total phenol content. Both the red and yellow *X. americana* fruit fleshs showed more than 90% of DPPH\* free radical scavenging. The total phenol content had significant effect for the higher antioxidant activity of the red *X. americana* fruit. The study also revealed that the seed part of *X. americana* fruit had higher total unsaturated fatty acid as compared to the flesh. Palmitic acid methyl ester was the major fatty acid detected in the flesh whereas elaidic acid, methyl ester was the dominant

fatty acid observed in the seed. The variations in nutritional and health imparting compounds might be explained by the genetic variability of the two colored fruits. This study confirmed that *X. americana* fruits grown in Ethiopia are rich in nutrients, phytochemicals contents and essential fatty acids. The fruits need to be fully studied to exploit its medicinal values to be used as functional foods.

#### Declarations

##### Author contribution statement

Asfawosen Mamo Bazezew: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shimelis Admassu Emire, Mulugeta Teamir Sisay: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### Data availability statement

Data will be made available on request.

##### Declaration of interests statement

The authors declare no conflict of interest.

##### Additional information

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