Phytochemicals and antioxidant activity of different apple cultivars grown in South Ethiopia: case of the wolyata zone

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ABSTRACT
Apple fruit is one of the most intensively grown and widely cultivated fruit crop in the temperate regions around the world. This study investigated sugar, vitamin C, total phenolic contents (TFC), total flavonoid contents (TFC), and antioxidant activity of three varieties (Golden delicious, Fuji rose, and Granny smith) of whole apple fruits (peel and flesh). Total sugar, reducing sugar and sucrose contents were determined by Lane and Enyon method. Folin-Ciocalteu and aluminum chloride, and standardized iodine solutions were used to determine TPC, TFC, and vitamin C, respectively. Antioxidant activity was determined using 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH) scaven- ging, total antioxidant and ferric ion reducing power assays. It was found that Golden delicious cultivar contained the highest amount of total sugar (17.32 ± 0.40 mg/100 g juice) and reducing sugar (10.04 ± 0.30 mg/100 g juice), whereas the juice of Fuji rose variety had the highest amount of sucrose (6.90 ± 0.35 mg/100 g of juice). The Granny Smith variety had the highest TPC (71.88 ± 2.30 mg gallic acid equivalent per gram of dried extract) whereas the Fuji rose had the highest TFC (21.91 ± 2.55-mg catechin equivalent per gram of dried extract). The Golden delicious cultivar had the highest vitamin C (31.48 ± 2.18 mg/100 g of juice). The Fuji rose showed the strongest DPPH radical scavenging activity (EC50 = 86.20 ± 2.28 μg/mL), iron reducing power (EC50 = 1.93 ± 0.66 mg/mL), and total antioxidant activity (0.46 ± 0.08 mg butylated hydroxy toluene equivalent/g of dried extract). Based on the present investigation, it can be concluded that the significant differences in the phytochemical composition and antioxidant activity of apple fruits could be attributed to varietal differences.

Introduction

Apple fruit (Malus Domestica) is a sweet, edible fruit produced by an apple tree in different regions of the world. The plant is an exogenous crop grown in the tropical climate of Ethiopia. The introduction of apple seedlings to Ethiopia is traced back to the 1950s to Chencha town in the Gamo Highlands of southwest Ethiopia and to Adigrat in Tigray, North Ethiopia. Growing apple is becoming an important horticulture activity in the highlands of Ethiopia, which help farmers to balance their diets, serve as cash crops to generate incomes, diversify production, conserve soil and environment and create employment opportunities for many households including youths.

Apples are a rich source of antioxidants, vitamins, minerals, phenolic compounds and also a good source of soluble carbohydrates such as starches, sugars and a fiber pectin, which helps to reduce cholesterol and blood glucose levels in humans. Among the health benefits, apples are used for treating breast cancer, Colon cancer, Alzheimer’s, Cardiovascular disease, and asthma. Environmental factors and varietal differences are the major factors affecting the phytochemical
compositions of apples in the manner in which they respond to different environmental conditions.\textsuperscript{[5]}

Therefore, this study was conducted to determine the sugar contents, vitamin C, total phenolic contents, and the antioxidant potential of ‘Golden delicious’, Fuji rose’, and ‘Granny smith’ apple varieties grown in South Ethiopia. Therefore, the objective of this study was to generate base-line information on the phytochemical composition and antioxidant activity of these apple cultivars.

\textbf{Material and methods}

\textbf{Chemicals}

Gallic acid, butylated hydroxy toluene (BHT), Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picylhydrazyl (DPPH), catechin, ascorbic acid, NaNO\textsubscript{2}, Na\textsubscript{2}CO\textsubscript{3}, AlCl\textsubscript{3}, potassium ferric cyanide, trichloroacetic acid, H\textsubscript{2}SO\textsubscript{4}, sodium phosphate, Iodine, KI, starch, methylene blue, and ammonium molybdate were purchased from Sigma-Aldrich (St.Louis, USA). The remaining chemicals were of analytical grade.

\textbf{Collection and preparation of apple juice}

The apple cultivars were collected from Bilate Charicho district, Duguna-Fango woreda, Wolaita zone, South Ethiopia. The varieties of ripen apple fruits (Golden Delicious, Fuji, and Granny Smith) were collected from the garden and then washed under distilled water to remove dust. The whole apple (flesh and peel) was cut into slices and the juice was prepared using Juicer. The samples were stored at 
\textdegree C until used for further analysis.

\textbf{Extraction of apple fruits}

A 20 g of the apple juice was placed in a 500 mL conical flask and dissolved with 100 mL 80\% methanol. The contents were kept in an orbital shaker for 12 h at room temperature. Thereafter, each extract was filtered using Whatman no.1 filter paper and evaporated to dryness under vacuum at 50\degree C by using a rotary evaporator (Buchi, 3000 series, Switzerland). The resulting extracts were stored in a sealed plastic container at 4\degree C until further investigation.

\textbf{Reducing sugar content}

Five grams of apple juice were weighed and transferred into 250 mL of volumetric flask according to the method of Aurelie \textit{et al.}\textsuperscript{[8]} with some modification. The solution was made up to 250 mL in a volumetric flask. Then, 50 mL of distilled water and 5 mL of each of Fehling A and B and 2 to 3 boiling chips were added in conical flask. Then, the content was boiled vigorously and the clarified solution taken in a burette was added while boiling until the blue color just disappears. 0.5 mL of methylene blue was added and allowed to boil for 3 min. While boiling, complete the titration as quickly as possible by adding 2–3 drops of juice at 5–10 seconds interval, until the indicator is completely decolorized and the brick-red color becomes dominant. Finally, the percentage of total reducing sugar was calculated as follows:

\[ \% \text{ of reducing sugar} = \frac{(0.05 \times 250 \times 100)}{TV \times W} \]

where, W is weight (g) of apple juice, TV (mL) is volume (mL) of diluted apple solution consumed, and \% of reducing sugar was expressed in terms of gram per 100 g of material (g/100 g).

\textbf{Total sugar content}

Juice (50 mL) (prepared for the estimation of reducing sugar) was pipetted in to a 250 mL conical flask. Five mL of dilute HCl solution and 50 mL of distilled water were added. The conversion of sucrose was boiled gently for 10 minutes to complete and then cooled. The content was transferred to a 250 mL
volumetric flask, neutralized with 1 N NaOH, and made up the volume to 250 mL. Then, the apple juice solution was titrated with 10 mL of Fehling solution. Percentage of total sugar was calculated by using the formula:

\[ \% \text{ of total sugar} = \frac{0.05 \times 250 \times 250 \times 100}{TV \times 50 \times W} \]

\[ \% \text{ of total sugar was expressed in terms of gram per 100 g of material (g/100 g).} \]

**Sucrose content**

The percentage of sucrose was calculated as follows:

\[ \% \text{ of sucrose content} = (TSC - RSC) \times 0.95 \]

Where TSC – Total Sugar Content, RSC – Reducing Sugar Content

**Vitamin C**

The vitamin C contents were determined according to the procedure described by Nweze et al.\[^9\] using redox titration method with slight modification briefly. 20 g of apple sample was chopped into small pieces and added into a 250 mL Erlenmeyer flask. Then, 50 mL of distilled water was added, filtered with cotton cloth and 1 mL of starch indicator solution was added. The sample was titrated with standardized iodine solution (0.005 mg/mL). The endpoint of the titration occurs when a permanent dark blue-black color appears due to the presence of the starch iodine complex. The vitamin C content of the samples was expressed in terms of mg/100 g of apple juice.

**Total phenolic content (TPC)**

The TPC was estimated by the Folin-Ciocalteu method,\[^10\] with slight modification using gallic acid as standard. Up to 0.1 mL of the extract (1 mg/mL), 1 mL Folin-Ciocalteu reagent (diluted 10 times) was added and the mixture was left for 5 min and then 1 mL (75 g/L) of sodium carbonate, Na\(_2\)CO\(_3\), was added. The absorbance of the resulting blue-colored solution was measured at 765 nm with a UV-visible spectrophotometer (JENWAY, 96500, UK) after incubation for 90 min at room temperature. The total phenolic content was estimated from the gallic acid (1–100 µg/mL) calibration curve (\(y = 0.024x - 0.014\), \(R^2 = 0.996\)) and results were expressed as mg gallic acid equivalent per gram of extract (mgGAE/g).

**Total flavonoid content (TFC)**

The TFC was estimated using the method of Ayoola et al.\[^11\] with a slight modification. The extract (1 mL, 1 mg/mL) was diluted with 1.25 mL distilled water and 75 µL 5% NaNO\(_2\) was added to the mixture. After 6 min, 150 µL 10% AlCl\(_3\) was added and after another 5 min, 1 mL 1 M NaOH was added to the mixture. Immediately, the absorbance of pink in color was determined at 510 nm versus prepared water blank. A standard curve was prepared using 5–1000 µg/mL of catechin. Results were expressed as milligram of catechin equivalents per gram of dried extract (mgCE/g). All the calculations were done using the standard equation obtained from the standard calibration curves of catechin (\(y = 0.0011x + 0.123\), \(R^2 = 0.97\)). The TFC in the crude extract was carried out in a triplicate experiment and the results were averaged.

**DPPH radical scavenging activity**

The DPPH radical scavenging activity of the extracts was determined as described by Engeda.\[^12\] Different concentrations (0.1–5 mg/mL) of the extracts were taken from different test tubes. A freshly prepared DPPH solution (2 mL, 0.06%, w/v) in methanol was added to each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference (ascorbic acid) were vortexed
and left to stand at room temperature in the dark for 30 min. Absorbance of the resulting solution was taken at 520 nm. Methanol was used as a blank. The ability of the DPPH radical scavenging (%) was calculated using the following equation.

\[
DPPH \text{ scavenging } (\%) = \left( \frac{AC - AS}{AC} \right) \times 100
\]

where Ac is the absorbance of the control and As is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as EC_{50} (effective concentration required to scavenge 50% of the DPPH).

**Ferric reducing power**

The reducing power was determined by assessing the ability of the extracts to reduce ferric ions as described by Abebie *et al.*[^13] The presence of antioxidants in the extract causes the reduction of the yellow ferric cyanide complex to the blue ferrous form, which can be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. One mL extract (final concentration 0.1–10 mg/mL) was mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide. Then, the mixture was incubated at 50°C for 20 min. Trichloro acetic acid (2.5 mL, 10%) was added to the mixture, which was then centrifuged at 3000 rpm (Centurion, 1000 series, UK) for 5 min. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL FeCl₃ (0.1%) and absorbance was measured at 700 nm. EC_{50} value, the effective concentration at which the absorbance was 0.5 nm for reducing capacity was obtained by interpolation from the graph of absorbance vs. concentration of apple extracts.

**Total antioxidant activity**

The total antioxidant capacity of the crude extracts was evaluated using the phosphor molybdenum method Engeda *et al.*[^14] with slight modification. The method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds or crude extract and subsequent formation of green Mo (V) complexes with a maximal absorption at 695 nm in the acidic medium. Plant extract (0.5 and 1 mg/mL) was mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Samples incubated at 95°C for 90 min were cooled at room temperature. The absorbance was also measured at 695 nm, and methanol (3 mL) was used as blank. The total antioxidant activity was expressed as milligram butylated hydroxy toluene equivalent/gram of extract (mg BHTE/g) based on the calibration curve (\(y = 0.0091x + 0.110, R^2 = 0.99\)).

**Statistical analysis**

Statistical analysis was performed using Statistical Analysis System (SPSS, Version 20). The data were subjected to analysis of variance (ANOVA). Duncan’s multiple-range test was used mean comparisons at \(p < .05\). Linear regression analysis was used to calculate EC_{50} values.

**Result and discussions**

**Sugar contents**

The total sugar content of Golden delicious was the highest followed by Fuji rose and Granny Smith cultivars (Table 1). There was no significant difference \((p > .05)\) in total sugar between Fuji rose and Golden delicious but they showed significantly higher total sugar content than that of Granny smith variety \((p < .05)\). The amount of reducing sugar in Golden delicious was the highest. The reducing sugar, sucrose, and total sugar contents of Golden delicious apple described by Ticha *et al.*[^15] were
lower than that reported in this study. Similarly, according to Lata,[16] the total sugar content of Golden delicious (13.6 g/100 g juice) was lower than that of reported in the present study (17.32 ± 0.4 g/100 g). The reducing sugar content of Fuji juice was lower but the total sugar and sucrose content were higher than that reported by Gilvan et al.[17]

**Vitamin C**

Vitamin C in the samples was determined by a redox titration using a standard iodine solution.[18] Vitamin C (ascorbic acid), is an essential antioxidant needed by the human body. As the iodine is added to apple juice during the titration, the ascorbic acid is oxidized to dehydroascorbic acid, while the iodine is reduced to iodide ions. Average concentrations of vitamin C in the sample are expressed in milligrams of ascorbic acid per 100 grams. Apple contains ascorbic acid, which serves as an antioxidant in addition to many other functions. Indeed, it has been shown that antioxidant activity of apple, which depends on its botanical origin, is related to its vitamin C contents, i.e., the content of vitamin C has a significant impact on total antioxidant activity of apple.[19] Table 2 represents the amount of vitamin C in the sample of apple juice ranged from 14.97 ± 1.28 to 31.48 ± 2.18 mg/100 g juice. Golden delicious showed the highest vitamin C content followed by Fuji rose. No significant difference (p > 0.05) was found between the vitamin C content of these two cultivars, but the vitamin C content of the Granny Smith sample was significantly lower (p < 0.05) than that of the other two samples. The vitamin C content of Fuji rose (27.12 mg/100 g juice) was higher but that of Granny smith lower than the results reported by Samira et al.[20] However, according to Robert's[21] report, vitamin C in Fuji rose and Granny Smith varieties was higher than the amount reported under this study.

**Total phenolic and flavonoid contents**

Phenolic compounds are generally considered as a very important antioxidant source in fruits. The total phenolic contents in the determination of apple fruit extracts using the Folin-Ciocalteu’s reagent were expressed in terms of milligram Gallic acid equivalent per gram (mg GAE/g). The three apple cultivar samples were investigated and exhibited considerable differences in their TPC values, varying from 66.94 ± 1.62 mg GAE/g for Golden delicious apple to 71.88 ± 2.30 mg GAE/g for Granny smith apple (Table 2). There was no significant difference (p > 0.05) in TPC between Granny smith and Fuji rose but these values were significantly higher (p < 0.05) than that of Golden delicious apple. According to the report of Pirlak et al.[22] TPC of these apple fruits was lower than that of the present

**Table 1.** Sugar contents of different varieties of apple cultivars.

| Sample          | Sucrose (g/100 g) | Reducing sugar (g/100 g) | Total sugar (g/100 g) |
|-----------------|-------------------|--------------------------|-----------------------|
| Golden Delicious| 6.76 ± 0.16<sup>b</sup> | 10.04 ± 0.30<sup>a</sup> | 17.32 ± 0.40<sup>b</sup> |
| Fuji Rose       | 6.90 ± 0.35<sup>b</sup> | 9.70 ± 0.10<sup>a</sup> | 17.10 ± 0.36<sup>b</sup> |
| Granny Smith    | 6.14 ± 0.10<sup>a</sup> | 9.71 ± 0.84<sup>a</sup> | 15.54 ± 0.27<sup>a</sup> |

Values are presented as mean ± SD of three determination values with different superscripts are along a column are significantly different from each other (p < 0.05).

**Table 2.** Total phenolic contents (TPC), total flavonoid contents (TFC) and vitamin C of different cultivars of apples.

| Samples          | TPC (mg GAE/g)* | TFC (mg CE/g)**| Vitamin C (mg/100 g) |
|------------------|----------------|----------------|----------------------|
| Golden delicious | 66.94 ± 1.62<sup>a</sup> | 15.59 ± 0.23<sup>a</sup> | 31.48 ± 2.18<sup>b</sup> |
| Fuji rose        | 70.90 ± 2.74<sup>b</sup> | 21.91 ± 2.55<sup>b</sup> | 28.83 ± 2.80<sup>b</sup> |
| Granny smith     | 71.88 ± 2.30<sup>b</sup> | 21.78 ± 1.87<sup>b</sup> | 14.97 ± 1.28<sup>a</sup> |

Where * and ** are total phenolic and total flavonoids expressed as gallic acid and catechin equivalents, respectively. Values are expressed as mean ± SD (n = 3) from triplicate experiments. Means with different letters in a column were significantly different at the level of p < 0.05.
study. These differences may be due to multiple reasons including genetic factors, different environmental conditions, storage of maturity, varietal differences, or soil fertilization. The highest TFC was found in Fuji rose, whereas the lowest one was measured in Golden delicious apple fruit (Table 2). The TFC in Fuji rose and Granny smith was not significantly different (p > 0.05). These values were significantly higher (p < 0.05) than that of Golden delicious variety. According to the report of Marinov et al., the TFC of Golden delicious and Granny smith were greater than that of the present study.

Antioxidant activity

DPPH scavenging activity
The DPPH is a stable free radical, which is scavenged by antioxidants through the donation of hydrogen forming the reduced DPPH. The color changes from purple 2, 2-diphenyl-1-picrylhydrazyl radical to reduced yellow diamagnetic 2, 2-diphenyl-1-picrylhydrazyl molecule, can be quantified by its absorbance reduction at wavelength 517 nm. As the concentration of samples increased, the percent inhibition of DPPH radicals also increased. At a concentration of 1 mg/mL, DPPH scavenging activity of ascorbic acid and sample extracts decreased in order of Ascorbic acid (96.34 ± 0.74%) > Granny smith (92.71 ± 0.33%) > Fuji rose (90.57 ± 0.22%) > Golden delicious (70.73 ± 1.49%) (Figure 1).

There is a reverse correlation between EC50 values and DPPH scavenging activity. The EC50 values of all the apple samples were calculated from the plotted graph of percentage scavenging activity against concentration of the apple extract (Figure 1). The lower the EC50 value, the higher is the scavenging potential. Fuji rose showed the strongest DPPH scavenging activity with the EC50 = 86, (20 ± 2.28 µg/mL) followed by Granny smith. Golden delicious showed significantly (p < 0.05) the weakest DPPH scavenging activity with EC50 value of 122.53 ± 3.48 µg/mL. No significant difference (p > 0.05) was observed between the IC50 values of Fuji rose and Granny smith samples. However, these values showed significantly stronger (p < .05) DPPH scavenging activity (Table 3). The DPPH radical scavenging activity of Fuji rose, Golden delicious, and Granny smith varieties in the present study were stronger than that of reported by Pirlak et al. and Karoline et al.

![Figure 1](image_url)

**Figure 1.** DPPH radical scavenging activity (%) of apple cultivars and control (ascorbic acid). Each value is expressed as mean ± standard deviation.
Ferric reducing power
This assay is based on a redox reaction in which antioxidants act as reductants and ferric ions act as oxidants. The increase in absorbance is directly correlated to the total ferric reducing power of the tested sample. The Fe³⁻-Fe²⁺ transformation was investigated in the presence of the apple extracts and the reducing capacity of compounds serves as a significant indicator of their potential antioxidant activity. All results revealed the iron reducing power in dose-dependent manner at concentrations of 0.1 to 10 mg/mL (Figure 2). At 10 mg/mL, the reducing power of apple fruit extracts and the reference (ascorbic acid) was found to decrease in this order of ascorbic acid (2.385 ± 0.80 nm) > Fuji rose (1.499 ± 0.34 nm) > Granny smith (0.869 ± 0.14 nm) > Golden delicious (0.429 ± 0.12 nm).

Similar to DPPH scavenging and total antioxidant results, the Fuji cultivar showed the strongest ferric reducing power (p < .05) with the EC₅₀ value of 1.93 ± 0.66 mg/mL, three times stronger than that of Granny smith and more than four times stronger than the ferric reducing power of Golden delicious variety (Table 3). Also, the ferric reducing power of Fuji rose was stronger than that of reported by Carolina et al.[30]

| Sample          | DPPH scavenging (µg/mL) | Ferric reducing (mg/mL) |
|-----------------|-------------------------|-------------------------|
| Golden delicious| 122.53 ± 3.48           | 8.84 ± 0.90             |
| Fuji rose       | 86.20 ± 2.28            | 1.93 ± 0.66             |
| Granny smith    | 93.25 ± 2.88            | 3.95 ± 1.98             |
| Ascorbic acid   | 40.04 ± 1.40            | 0.58 ± 0.07             |

Values are average of triplicate measurements (mean ± SD). Mean value with different superscripts in the column are significantly different (p < 0.05).

Total antioxidant activity
The results of Figure 3 revealed that Fuji rose apple at 5 mg/mL showed the strongest total antioxidant activity (0.46 ± 0.08 mg BHTE/g) and the weakest total antioxidant activity (0.30 ± 0.03 mg BHTE/g) was found in the Granny smith. A significant difference (p < 0.05) was found between the total

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Figure 2. Ferric reducing power of apple cultivars and control (Ascorbic acid); values are average of triplicate measurements (mean ± SD).
antioxidant activity of Golden delicious (0.33 ± 0.01 mg BHTE/g), Fuji rose (0.46 ± 0.08 mg BHTE/g) and Granny smith (0.30 ± 0.03 mg BHTE/g). Similarly, at a concentration of 2.5 mg/mL, Fuji rose showed a stronger ($p < 0.05$) total antioxidant (0.11 ± 0.04 mg BHTE/g) than that of Golden delicious and Granny smith with values of 0.08 ± 0.01 and 0.071 ± 0.004 mg BHTE/g, respectively.

According to Pirlak et al.\cite{22} report, the total antioxidant activity of Fuji rose was found to be 0.458 mg BHTE/g, similar to the result determined in the present study (0.46 ± 0.08 mg BHTE/g). Similarly, the total antioxidant activity of Granny smith (0.30 mg BHTE/g) was in agreement with the report of Giomaro et al.\cite{31} but the total antioxidant activity of Fuji rose was stronger than the total antioxidant activity reported by these researchers.

**Conclusion**

Apples are one of the most produced, exported and consumed fruits in the world. The health benefits of apples are mainly attributed to their phenolic content. The results of this study support that these apple cultivars are promising sources of natural antioxidants. The phenolic content and antioxidant capacity differ significantly among all cultivars. The Fuji rose cultivar has the highest total flavonoid content. This cultivar exhibited the strongest DPPH radical scavenging, iron reducing power, and total antioxidant capacity. This suggests that the antioxidant activity of the tested extracts was closely associated with their phenolic constituents. The study also revealed that these apple cultivars contain a considerable amount of phenolic compounds, which can be used as easily accessible sources of natural antioxidants and as a possible food supplement or in pharmaceutical applications.

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