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ASCORBIC ACID CONTENT AND THE ANTIOXIDANT ACTIVITY OF COMMON FRUITS COMMERCIALLY AVAILABLE IN ADDIS ABABA, ETHIOPIA

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ABSTRACT. The present work was aimed to determine the contents of ascorbic acid and the antioxidant activity of eight types of common fruits commercially available in Addis Ababa, Ethiopia using iodometric titration and UV-Vis spectrophotometric methods, respectively. The contents of ascorbic acid were found in the range 15.41 mg/100 g in grapes to 78.01 mg/100 g in papaya. The antioxidant activity was found highest in papaya (89.41) and lowest in water melon (31.28) in mg of ascorbic acid equivalent per 100 g of the edible part of fruit juice. The calibration curve of ascorbic acid was in the range 50 - 3.125 mg/L with $R^2 = 0.9994$. The results also revealed the strong correlation (r = 0.863) between ascorbic acid contents in fruits and their antioxidant activity. Using the optimized conditions, UV-Vis spectrophotometric method with the 2,2-diphenyl-1-picrylhydrazil assay allows determination of antioxidant activity of fruits with limit of detection 0.32 mg/L and limit of quantification 0.96 mg/L. The precision was in the acceptable range (relative standard deviation, RSD < 20%) for determination of ascorbic acid and < 10% for antioxidant activity determination. The recovery of ascorbic acid was between 85% and 113% for selected fruits showing good accuracy of the results.

KEY WORDS: Fruits, Ascorbic acid contents, Antioxidant activity, Free radical scavengers, Iodometric titration, UV-Vis spectrophotometry

INTRODUCTION

Fruits and vegetables are excellent sources of antioxidants, vitamins, minerals, water, carbohydrates, fats, proteins, fiber, organic acids and pigments that stimulate our body and can help to offset certain diseases and help to be healthy [1, 2]. According to Nweze *et al.* [3] fruit is a part of flowering plant that derives from specific tissues of the flower, one or more ovaries and in some cases accessory tissues. Fruits account for a substantial fraction of a world's agricultural output that becoming a good source of food. Vitamin C is one of the major components of essential nutrients in fruits. Vitamin C is a water-soluble vitamin that is essential for all humans and a few other mammals [4-6].

Vitamin C is abundantly available in many natural sources, including fresh fruits and vegetables. The richest sources of ascorbic acid include fruits like Indian gooseberry, oranges, lemons, papaya, kiwifruit, strawberries and cantaloupes; green leafy vegetables such as broccoli, cabbage, brussels sprouts, bean sprouts, cauliflower, kale, mustard greens, red and green peppers, peas, potatoes, and fortified cereals. Animals such as ancestor of vertebrates, fishes, amphibians, and reptiles synthesize their own vitamin C and are highly concentrated in the liver part. But limited number of mammalian species, such as the guinea pigs, humans, apes, prosimian tarsiers, monkeys and bats are defective to synthesize vitamin C, due to a lack of L-gulonolactone oxidase (GULO) [7-9]. As an antioxidant, vitamin C reduces the risk of arteriosclerosis, cardiovascular diseases, infectious diseases, asthma, cataract, *Diabetes mellitus* and some forms of cancer [10]. It contributes prevention of scurvy, relief from common cold, play a significant role in wound healing process. It also inhibits free radicals from being oxidized in preventing cell damage and commonly used as food additive [11].

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Deficiency of vitamin C results in different health effects such as scurvy (spots on and bleeding under the skin, spongy gums, 'corkscrew' hair growth, poor wound healing) and unstable collagen. In advanced scurvy there are open, suppurating wounds, loss of teeth, bone abnormalities and, eventually, death [12]. A total body pool of vitamin C less than 300 mg is associated with scurvy and other disease symptoms, while maximum body pools are limited to about 2 g. With large intakes of vitamin C (above 2 g); unabsorbed ascorbate is degraded in the intestine, account for the diarrhea. Some of its metabolites like oxalic acid, inhibits of natural processes, deteriorates the taste/aroma of food and beverages; and also results in renal problems, nausea and gastric irritation [13, 14].

For optimal physiological conditions, the level of oxidants and antioxidants should be balanced. According to Sun *et al.* [15] over production of oxidants results in damage of larger biomolecules such as lipids, DNA, proteins which in turn leads to cancer. So this must be balanced with dietary antioxidants which are obtained mainly from fruits and vegetables. Antioxidants are substances that prevent the formation of free radicals by removing their intermediates; and they inactivate reactive oxygen species. They are not produced in sufficient quantities by the body, especially after a certain age. So it is important to get it through intake of fresh fruits and vegetables [16, 17]. Fruits and vegetables contain many different antioxidant components. The majority of the antioxidant components include vitamin C, vitamin E or β-carotene. Some flavonoids (including flavones, isoflavones, flavonones, anthocyanin, catechin and isocatechin), phenols and polyphenols are also a group of antioxidant. Synthetic antioxidants (butylated hydroxyl anisole-BHA, butylated hydroxyl toluene-BHT) play a useful role in food and pharmaceutical industries and other antioxidants include minerals (Se, Mn, Cu, Zn) [16, 17]. Antioxidants are important in stimulating, maintaining health and enabling the body to deal with attacks from both external and internal environment. The natural immune system can fight and neutralize aggression more quickly and thus prevent imbalance diseases, inflammation, degenerative processes, aging and even activation and abnormal cell replication (typical cancers) and cardiovascular diseases [16, 18].

The relation between ascorbic acid content and antioxidant capacity in vitro is investigated in order to identify the percentage contribution to the antioxidant activity of fruits [18]. Many methods were developed and reported in the literature for the determination of the contents of ascorbic acid in its sources; such as titrimetric, spectrophotometry, high performance liquid chromatography (HPLC) and voltammetry methods [4, 6, 11, 13, 19]. However, some of these methods are time-consuming, some are costly, some need special trained operators and others suffer from the insufficient sensitivity or selectivity [10]. The most commonly used methods are titrimetric and spectrophotometric methods. Titration is used for the determination of ascorbic acid content in most literatures since it is easy. The oxidation reduction reaction is better than an acid-base titration since there are additional acids in the juice, but few of them interfere with the oxidation of ascorbic acid by iodine. Iodine is relatively insoluble, but this can be improved by complexing the iodine with iodide to form tri-iodide:

$$I_2 + I^- \rightleftharpoons I_3^-$$

Tri-iodide oxidizes ascorbic acid to form dehydroascorbic acid:

$$C_6H_8O_6 + I_3^- \longrightarrow C_6H_6O_6 + 3I^- + 2H^+$$

As long as ascorbic acid is present in the solution, the tri-iodide is converted to the iodide ion very quickly. However, when all the ascorbic acid is oxidized, iodine and tri-iodide will be present, which react with starch to form a blue-black complex that indicates the endpoint of the titration. This titration procedure is appropriate for testing the amount of ascorbic acid in vitamin C tablets, juices, and fresh, frozen, or packaged fruits and vegetables which was performed using just iodine solution [3].

It is also important to determine the antioxidant activity of fruits. There are different assays that are used to measure the antioxidant capacity of fruits. This include 2,2-diphenyl-1-picrylhydrazil radical (DPPH) assay, trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP), oxygen radical absorbance capacity (ORAC) and cupric reducing antioxidant capacity (CUPRAC) assays can be described [16-18, 20-22]. DPPH assay is the most common because it is a rapid, simple and inexpensive assay. DPPH is used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. It is based on the reduction of DPPH stable free radical and an odd electron gives a maximum absorption at 517 nm with color less, pale yellow or purple color [20, 23].

According to Amarowicz and Pegg [22] the DPPH assay is often run due to its relative inexpensiveness which undergoes hydrogen atom transfer (HAT) and single electron transfers (SET) or mixed mechanism of the two. The hydrogen atom transfer occurs when an antioxidant compound quenches free radical species by donating H-atom. The free radical formed in this reaction is much more stable than RO_2 radical.

$$DPPH_{(violet\ at\ 517\ nm)} + ArOH \longrightarrow DPPH(H)_{(colorless)} + ArO^{\cdot}$$

The single electron transfers mechanism occurs in cases where an antioxidant transfers a single electron to aid in the reduction of potential target compounds.

$$DPPH_{(violet\ at\ 517\ nm)} + ArOH \longrightarrow DPPH^{-}_{(colorless)} + ArO^{\cdot+}$$

The resultant radical cationic antioxidant compound is then deprotonated by interacting with water.

$$[ArOH]^{\cdot+} + H_2O \rightleftharpoons ArO^{\cdot} + H_3O^{+}$$

$$RO_2^{-} + H_3O^{+} \rightleftharpoons ROOH + H_2O$$

HAT and SET chemical processes can occur simultaneously as a sequential proton loss electron transfer (SPLET), which also termed as proton coupled electron transfer (PCET).

$$ArOH \longrightarrow ArO^{-} + H^{+}$$

$$ArO^{-} + ROO^{\cdot} \longrightarrow ArO^{\cdot} + ROO^{-}$$

$$ROO^{-} + H^{+} \rightleftharpoons ROOH$$

There is no enough study about the antioxidant activity of fruits in Ethiopia. This study was intended to determine, compare and contrast the ascorbic acid contents and its antioxidant activity in fruits by iodometric titration method and UV-Vis spectrophotometry method (DPPH assay), respectively. This study aware the health benefit of fruits in accordance with ascorbic acid and their antioxidant activity. This study can also be used as starting point for further studies about the content of ascorbic acid in fruits and the health benefit of fruits. The generated data and information on the ascorbic acid chemistry and its antioxidant activity in the fruits can be used as a baseline information for future researches on the investigation of the content of ascorbic acid and the antioxidant behavior of common fruits in Ethiopia.

EXPERIMENTAL

Instruments and chemicals

In this research double beam spectrophotometer (Lambda 950 – UV-Vis-NIR, Perkin Elmer, UK) interfaced with a computer using 2 nm resolution in a 1 cm path length quartz cell, digital balance (model PW 254) with precision 0.0001 g and centrifuge (80-2, China) were used. KIO_3, KI,

L-ascorbic acid (Riede-de Haen), DPPH (2,2-diphenyl-1-picrylhydrazyl), 99.8% methanol (Himedia, India), starch indicator and 96% H_2SO_4 (Carlo Erba) were used.

Preparation of reagents and standards

Preparation of 3 M H_2SO_4. Aliquots of 16.735 mL of 96% of H_2SO_4 and 83.265 mL distilled water were mixed in 100 mL volumetric flask and 100 mL 3 M H_2SO_4 was prepared.

Preparation of 0.5% starch indicator solution. Exactly 0.125 g of soluble starch was dissolved in 25 mL near boiling distilled water (70 ºC) in 25 mL volumetric flask. Then it was mixed well and allowed to cool before use.

Preparation of iodine solution. A 2.5 g of potassium iodide (KI) and 0.134 g of potassium iodate (KIO_3) were weighed and dissolved in 100 mL of distilled water in 250 mL volumetric flask. Then 15 mL of 3 M H_2SO_4 was poured in the solution. The obtained solution was diluted to a final volume of 250 mL with distilled water and was mixed well by shaking the solution.

Preparation of ascorbic acid standard (60 mg/L) solution for titration. A 60 mg/L of ascorbic acid standard solution was prepared by dissolving 0.0060 g of L-ascorbic acid in 25 mL distilled water and then diluted to 100 mL with distilled water in 100 mL volumetric flask.

Standardizing iodine solutions. A 20 mL of 60 mg/L ascorbic acid standard solution was taken to a 100 mL Erlenmeyer flask and 1.5 mL of 0.5 % starch solution was added. This solution was titrated with iodine solution until the endpoint was reached, characterized by the first sign of appearance of blue black color that persists after swirling the solution. Triplicate titration was conducted and then the mean was taken for the reliability of the result.

Preparation of DPPH and standard ascorbic acid solution for antioxidant activity

The antioxidant activity (AOA) of different fruit sample extracts were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method reported by Haile *et al.* [24] with some modifications. A mass of 0.016 g of DPPH was dissolved with little amount of methanol in a 100 mL volumetric flask. After the DPPH fully dissolved, the flask was filled up to the mark using methanol to get a concentration of 160 mg/L solution. The control was measured by using the mixture of 4 mL methanol and 2 mL DPPH solution. Besides, a stock solution of ascorbic acid (200 mg/L) was prepared by dissolving 0.02 g of ascorbic acid in 100 mL of volumetric flask using methanol.

Sample collection and preparation

Eight types of common fruits; orange (*Citrus sinensis*), apple (*Malus domestica*), pineapple (*Ananas comosus*), watermelon (*Citrullus lanatus*), lemon (*Citrus lemon*), grapes (*Vitis vinifera*), mango (*Mangifera indica*) and papaya (*Carica papaya*) were randomly purchased from local markets in Addis Ababa, Ethiopia and taken to laboratory in polyethylene plastic bags. The fruits were fresh, ripen and ready to eat. The analysis was carried out in 24 hours of fruits collection time in analytical chemistry laboratory in Chemistry Department, Addis Ababa University, Ethiopia.

The method of sample preparation was adopted from Songsermsakul *et al.* [25] with some modifications. In detail, all fruits were thoroughly washed with tap water to remove dusts and unwanted particles and weighed. The edible parts of the fruits were cut and hand squeezed (orange and lemon) and crushed (watermelon, grapes, apple, pineapple, mango and papaya) with mechanical crusher. The obtained juice was filtered with clean cloth and weighed for comparison

purpose. From the obtained juice, 20 mL aliquot of the juice was measured and diluted up to 100 mL with deionized water in 100 mL volumetric flask. The diluted solution was mechanically shaken with hand for 10 min, centrifuged for 20 min at 3000 rpm (revolution per min) and was filtered with Whatman No. 41 filter paper.

Determination of ascorbic acid by iodometric titration

The method for the determination of ascorbic acid was adopted from Nweze *et al.* [3] with some modifications. It is based on iodometric titration of the sample with standardized iodine solution. To determine the amount of ascorbic acid in fruits, 20 mL of the clear solutions of the fruits were taken and 1.5 mL of 0.5% starch solution was added to each extract. These solutions were titrated against the prepared iodine solution (0.00724 M) with continuous shaking and the end point was recorded for all fruits. The end point of the titration was taken at the first sign of appearance of blue black color that persists on swirling. Triplicate titration was conducted and the mean was taken.

Determination of antioxidant activity of fruits by DPPH assay

The method of analysis for the determination of antioxidant activity of fruits was adopted from Haile *et al.* [24] with some modifications. From each sample extract, a 1 mL volume of the sample extract was mixed with 4 mL methanol and 2 mL of 160 mg/L DPPH solution in flasks and covered with aluminium foil and laboratory film. The mixture was incubated in the dark at room temperature for 60 min and then the absorbance was recorded at 517 nm. Each sample was analyzed in triplicate and the mean was taken.

Calibration curve. A calibration curve was established by preparing different concentrations of standard ascorbic acid, 50.0, 25.0, 12.5, 6.25 and 3.125 mg/L, from the stock solution. From each standard solution of ascorbic acid a volume of 1 mL was transferred in to five different 25 mL volumetric flasks and to each flask 4 mL of methanol and 2 mL of DPPH solution was added and then the solution was incubated in the dark at room temperature for about 60 min. Finally, the absorbance of all the standards was taken at wavelength of 517 nm to construct the calibration curve.

LOD and LOQ. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by measuring the absorbance of the control six times. Then, taking the standard deviation (SD) of these values and the slope of the calibration curve, the following equations were used [30].

$$\text{LOD} = \frac{3.3\ \text{SD}}{\text{Slope of calibration curve}}$$

$$\text{LOQ} = \frac{10\ \text{SD}}{\text{Slope of calibration curve}}$$

RESULTS AND DISCUSSION

The percentage of the juice in fruits

The fruits and their corresponding juice were weighed after squeezing the edible part of each fruits. Papaya (72.34%) has the highest and mango (31.08%) has the lowest percentage of juice compared to the other fruits. Table 1 shows the percentage of the juice obtained from the eight types of fruits.

Table 1. Percentage of the fruit juice.

No.	Sample	Weight of fruit taken (g)	Amount of juice obtained (g)	Percentage of fruit juice (%)
1	Apple	47.53	26.38	55.51
2	Grapes	150.3	102.0	67.88
3	Lemon	74.00	40.08	54.16
4	Mango	205.8	63.97	31.08
5	Orange	116.2	58.29	50.16
6	Papaya	120.6	87.26	72.34
7	Pineapple	75.90	45.74	60.26
8	Water melon	134.5	88.84	66.05

Determination of the contents of ascorbic acid in fruits

The contents of ascorbic acid in eight types of common fruits were determined by iodometric titration method as shown in Table 2. Highest amount of ascorbic acid was obtained in papaya (78.01 mg/100 g) and the lowest amount was found in grapes (15.41 mg/100 g). To determine the precision of the data triplicate analysis was conducted then the standard deviation (SD) and RSD of the data were calculated. A RSD below 20% is considered to be acceptable, below 10% is good precision and below 5% shows best precision of the obtained data [26]. In the present study only two samples water melon (16.9%) and apple (17.3%) revealed poor precision but within the accepted range. The other two, lemon (6.18%) and mango (6.18%) have shown good precision of the result. The rest four fruits have RSD less than 5% which have indicated the best precision of the data.

Table 2. Content of ascorbic acid in the fruits determined by iodometric titration.

No.	Sample	Concentration (mg/100 g)				SD
		Trial 1	Trial 2	Trial 3	Mean	
1	Apple	18.91	25.22	18.91	21.01	3.64
2	Grapes	15.41	15.41	15.41	15.41	0.03
3	Lemon	49.35	54.83	49.35	51.18	3.16
4	Mango	30.41	27.37	27.37	28.38	1.76
5	Orange	51.10	51,1	51.10	51.10	0.05
6	Papaya	77.00	77.00	80.02	78.01	1.74
7	Pineapple	53.90	50.90	53.90	52.90	1.73
8	Water melon	15.20	18.34	21.39	18.31	3.10

Comparison of the contents of ascorbic acid in fruits by titration and other methods between the present and previous studies is shown in Table 3. The content of ascorbic acid in lemon in the present study is almost similar to values reported by Najwa and Azrina [11] (44.0 ± 0.93 mg/100 g), Tee *et al.* [27] (46.8 ± 0.0 mg/100 g) and Desai and Desai [12] (56.4 mg/100 g), but slightly different with that reported by Porto *et al.* [28] (25.0 ± 2.8 mg/100 g). Generally, there were some results similar to the present study, but also some studies that show significant difference with the present study. This variation may have resulted from the difference in conditions such as pre-harvest system, handling, exposure to light, utilization of organic fertilizer, ripening stage, storage temperature, irrigation system of fruits and so on [11, 29].

Table 3. Comparison of ascorbic acid contents in fruits between the present study and the previous reported values.

No.	Sample	Present study	Najwa and Azrina [11]		Porto *et al.*[28] (digital image colorimetry)	Tee *et al.* [27] (dye titration)	Desai and Desai [5] UV-Vis spectrophotometric
			Titration	HPLC			
1	Apple	21.01 ± 3.64	-	-	-	5.1 ± 0.2	18.89
2	Grapes	15.41 ± 0.00	49.2 ± 0.53	26.4 ± 0.12	4.7 ± 0.6	2.9 ± 0.0	5.58
3	Lemon	51.18 ± 3.16	44.0 ± 0.93	31.3 ± 0.92	25.0 ± 2.8	46.8 ± 0.0	56.4
4	Mango	28.38 ± 1.76	-	-	32.8 ± 3.1	56.5 ± 1.9	36.41
5	Orange	51.10 ± 0.00	58.3 ± 0.53	43.6 ± 1.7	35.4 ± 1.3	42.7 ± 1.5	-
6	Papaya	78.01 ± 1.74	-	-	-	67.9 ± 0.6	-
7	Pineapple	52.90 ± 1.73	-	-	5.4 ± 0.3	14.3 ± 1.0	-
8	Water melon	18.31 ± 3.10	-	-	-	5.2 ± 0.0	9.79

Determination of the antioxidant activity of fruits

Calibration curve. The standard calibration curve is presented in Figure 1. The overlay of the spectra of the standards at a wavelength of 517 nm is shown in Figure 2.

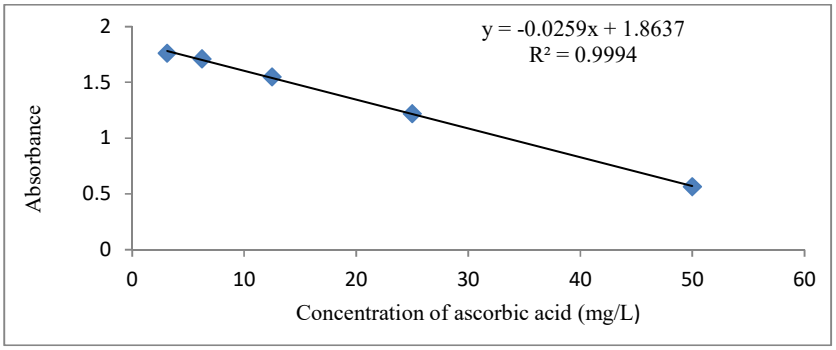

Figure 1. The calibration curve of ascorbic acid standard by DPPH scavenging activity.

The quantitative determination of the antioxidant activities was carried out based on the linear correlation between absorbance and concentration of ascorbic acid in DPPH solution. As can be seen in Figure 1, the calibration curve revealed negative correlation with R^2 value of 0.9994 but shows best linearity of the result. This is because as the concentration of ascorbic acid increases the absorbance decreases, in that manner concentration of the DPPH solution decreased, since it was converted to DPPH-H by taking one hydrogen atom from ascorbic acid.

UV-Vis spectra for samples

The UV-Vis spectra of antioxidant activity of fruits in aqueous solvent extraction by DPPH assay shows a maximum wavelength at (λ_{max} = 517 nm) which are presented in Figure 3 by overlaying the spectra.

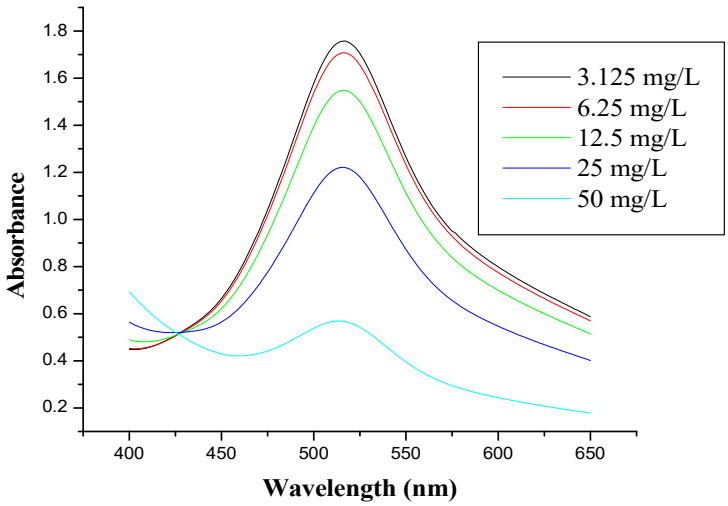

Figure 2. The UV-Vis spectra of the standard ascorbic acid.

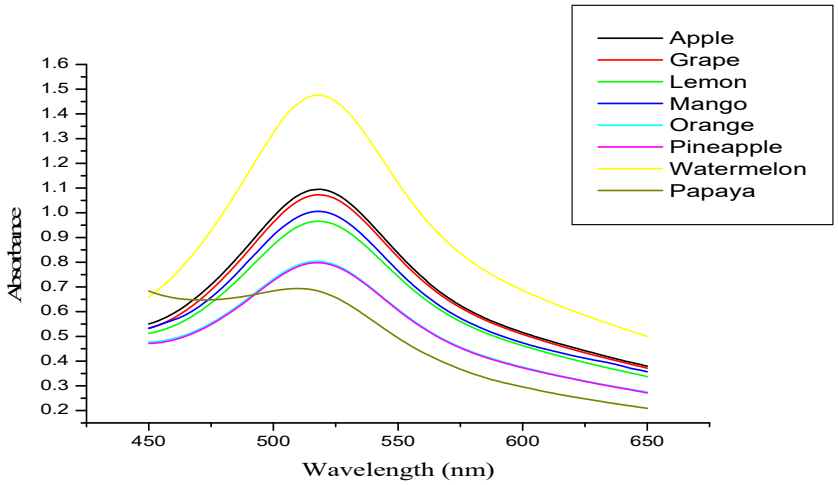

Figure 3. The UV-Vis spectra of the eight types of common fruits.

Amount of antioxidant activity determined

After the samples were analysed by DPPH assay with UV-Vis spectrophotometry and calculating the antioxidant activity of fruits from the calibration curve equation (since absorbance is inversely proportional to antioxidant activity of fruits), the following results were obtained as shown in Table 4. The highest amount of antioxidant activity was obtained in papaya (89.41 mg/100 g) and the lowest amount was in water melon (31.28 mg/100 g). To determine the precision of the data

triplicate analysis was conducted and then the SD and RSD of the data were calculated. In the present study only one sample mango (8.57%) which was above 5% RSD shows good precision of the result. The other seven fruits have RSD less than 5% which shows the best precision of the data.

Table 4. Antioxidant activity of the fruits using DPPH assay by UV-Vis spectrophotometry.

No.	Sample	Absorbance					AOA
		Trial 1	Trial 2	Trial 3	Mean	SD	(mg/100 g)
1	Apple	1.0911	1.0948	1.0834	1.090	0.006	55.92
2	Grapes	1.0850	1.0770	1.0727	1.078	0.006	57.65
3	Lemon	0.9309	1.0232	0.9659	0.973	0.047	66.12
4	Mango	1.1540	0.9913	1.0057	1.050	0.090	60.02
5	Orange	0.7975	0.7503	0.8040	0.784	0.029	78.32
6	Papaya	0.6918	0.6513	0.6483	0.664	0.024	89.41
7	Pineapple	0.7516	0.8101	0.7981	0.787	0.031	80.00
8	Water melon	1.3980	1.4390	1.4760	1.438	0.039	31.28

LOD, LOQ and recovery

The LOD and LOQ were found to be 0.32 mg/L and 0.96 mg/L, respectively (Table 5). The efficiency of the analyte extraction from the sample was checked by a recovery test. The recovery tests were done for both parameters for selected fruits by spiking a known amount of ascorbic acid to the sample extract. For antioxidant activity by DPPH assay lemon sample give a recovery of 98.66%. For ascorbic acid by iodometric titration gives a recovery of lemon (85%), grape (85%) and papaya (113%) which shows effective extraction of the sample in both cases.

Table 5. Result of LOD and LOQ.

Trials	1	2	3	4	5	6	SD	Slope	LOD (mg/L)	LOQ (mg/L)
Absorbance	1.5227	1.5254	1.5259	1.5275	1.5287	1.5295	0.0025	-0.0259	0.32	0.96

Table 6. Relationship between ascorbic acid contents and its antioxidant capacity in fruits.

No.	Sample	AOA (mg/100 g)	Ascorbic acid (mg/100 g)	Percentage (%)
1	Apple	55.92	21.01	37.58
2	Grapes	57.65	15.41	26.73
3	Lemon	66.12	51.18	77.40
4	Mango	60.02	28.38	47.29
5	Orange	78.32	51.10	65.25
6	Papaya	89.41	78.01	87.25
7	Pineapple	80.00	52.90	66.13
8	Water melon	31.28	18.31	58.55

Relationship between content of ascorbic acid and its antioxidant activity in fruits

The value of the percentage between the contents of ascorbic acid and the antioxidant activity of fruits was between the highest percentage of 87.25% for papaya and the lowest percentage was 26.73% for grape. There was a direct relationship between the contents of ascorbic acid and antioxidant activity of fruits. The higher content of ascorbic acid resulted in fruits with higher antioxidant activity. Using simple linear correlation, the relation between ascorbic acid and

antioxidant activity of fruits was performed by Pearson correlation. There was a strong correlation between content of ascorbic acid and antioxidant activity of fruits (r = 0.863) (Figure 4). This relation indicates the antioxidant activity in fruits is mainly contributed by ascorbic acid (Table 6).

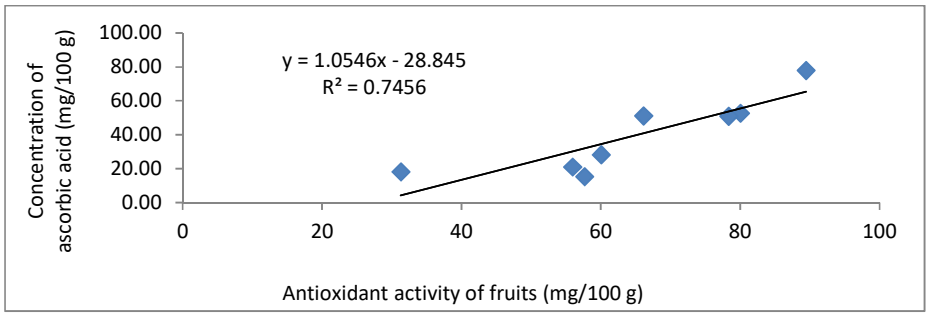

Figure 4. Relationship between ascorbic acid contents and antioxidant capacity of fruits.

CONCLUSION

In the present study eight types of fruits commonly consumed in Ethiopia were analysed for their ascorbic acid contents and antioxidant activity. The sample extraction method employed was aqueous extraction which was successful for both determinations. The ascorbic acid contents were determined by iodometric titration and the result was comparable with other reported values. With the same extraction procedure the obtained solution was analysed using UV-Vis spectrophotometry method by DPPH assay for determination of antioxidant activity of fruits. The limit of detection has assured best sensitivity of the method, accuracy and precision results have confirmed the method is really reproducible and repeatable. The ascorbic acid content of the fruits and antioxidant activities determined have shown very strong correlation. Among the eight common fruits, papaya has the highest value for both ascorbic acid content and antioxidant activity. Hence, the findings of this study reflected that the consumption of any of the eight types of the fruits is recommended for healthy life.

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REFERENCES

1. Vicente, A.R.; Manganaris, G.A.; Sozzi, G.O.; Crisosto, C.H. *Nutritional Quality of Fruits and Vegetables*, Elsevier: Amsterdam; **2009**; pp. 58-106.
2. Mihalache-Arion, C. The antioxidant capacity of vegetables and fruits. *Lucrări Ştiinţifice – Seria Agronomie* **2014**, 52, 545-550.
3. Nweze, C.C.; Abdulganiyu, M.G.; Erhabor, O.G. Comparative analysis of vitamin C in fresh fruits juice of *Malus domestica*, *Citrus sinensi*, *Ananas comosus* and *Citrullus lanatus* by iodometric titration. *Int. J. Sci. Environ. Technol.* **2015**, 4, 17-22.

4. Elgailani, I.E.H.; Gad-Elkareem, M.A.M.; Noh, E.; Adam, O.E.-A.; Alghamdi, A.M.A. Comparison of two methods for the determination of vitamin C (ascorbic acid) in some fruits. *Am. J. Chem.* **2017**, 2, 1-7.
5. Devaki, S.J.; Raveendran, R.L. *Chapter 1, vitamin C: Sources, functions, sensing and analysis.* Chemical Sciences and Technology Division, National Institute for Interdisciplinary Science and Technology: India; **2017**; pp. 3-20.
6. Devolli, A.; Stafasani, M.; Shahinasi, E.; Dara, F.; Hamiti, H. Determination of vitamin C content in commercial fruit juices by volumetric and spectrophotometric methods. *J. Hyg. Eng. Des.* **2021**, 124-131.
7. WHO Library Cataloguing-in-Publication Data. *Vitamin and mineral requirements in human nutrition*, 2nd ed., Report of a joint FAO/WHO expert consultation: Bangkok, Thailand; **1998**.
8. Cui, J.; Pan, Y.-H.; Zhang, Y.; Jones, G.; Zhang, S. Progressive pseudogenization: Vitamin C synthesis and its loss in bats. *Mol. Biol. Evol.* **2011**, 28, 1025-1031.
9. Akram, M.; Munir, N.; Daniyal, M.; Egbuna, C.; Găman, M.-A.; Onyekere, P.F.; Olatunde, A. *Chapter 9, Vitamins and minerals: Types, sources and their functions*. Functional Foods and Nutraceuticals; **2020**; pp. 149-172.
10. Bekele, D.A.; Geleta, G.S. Iodometric determination of the ascorbic acid (vitamin C) content of some fruits consumed in Jimma Town Community in Ethiopia. *Res. J. Chem. Sci.* **2015**, 5, 60-63.
11. Najwa, R.F.; Azrina, A. Comparison of vitamin C content in citrus fruits by titration and high performance liquid chromatography (HPLC) methods. *Int. Food Res. J.* **2017**, 24, 726-733.
12. Desai, A.P.; Desai, S. UV-spectroscopic method for determination of vitamin C (ascorbic acid) content in different fruits in South Gujarat Region. *Int. J. Environ. Sci. Nat. Resour.* **2019**, 21, 41-44.
13. Musara, A. *Determination of ascorbic acid in some citrus fruits by using pencil graphite electrode*. MSc Thesis, Near East University, Nicosia, **2019**.
14. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. National Academy Press: Washington, D.C.; **2000**.
15. Sun, J.; Chu, Y.F.; Wu, X.; Liu, R.H. Antioxidant and anti-proliferative activities of common fruits. *J. Agric. Food Chem.* **2002**, 50, 7449-7454.
16. Wang, H.; Cao, G.; Prior, R.L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, 44, 701-705.
17. Prakash, D.; Upadhyay, G.; Pushpangadan, P.; Gupta, C. Antioxidant and free radical scavenging activities of some fruits. *J. Complement. Integr. Med.* **2011**, 8, Article 23.
18. Proteggente, A.R.; Pannala, A.S.; Paganga, G.; Buren, L.V.; Wagner, E.; Wiseman, S.; Put, F.V.D.; Dacombe, C.; Catherine, A.; Rice-Evansa, C.A. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radic. Res.* **2002**, 36, 217-233.
19. Sharma, H.; Sapkota, H.P.; Khanal, A.; Dhakal, O.; Gurung, R. A comparative analysis of vitamin C concentration in commercial fruit juices and fresh fruits of Nepal with effect of temperature. *Int. J. Pharm. Pharm. Sci.* **2019**, 11, 46-51.
20. Shekhar, T.C.; Anju, G. Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. leaves. *Am. J. Ethnomed.* **2014**, 1, 244-249.
21. Rahman, M.M.; Islam, M.B.; Biswas, M.; Alam, A.H.M.K. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *J. Biomed. Sci.* **2015**, 8, Article 621.

22. Amarowicz, R.; Pegg, R.B. Natural antioxidants of plant origin. *Adv. Food Nutr. Res.* **2019**, 90, 1-81.
23. Nithya, P., Madhavi, C. Antioxidant studies of 3-arylidene-4-piperidones by DPPH assay. *J. Taibah Univ. Sci.* **2014**, 11, 40-45.
24. Haile, K.; Mehari, B.; Atlabachew, M.; Chandravanshi, B.S. Phenolic composition and antioxidant activities of cladodes of the two varieties of cactus pear (*Opuntia ficus-indica*) grown in Ethiopia. *Bull. Chem. Soc. Ethiop.* **2016**, 30, 347-356.
25. Songsermsakul, P.; Pornphairin, E.; Porasuphatana, S. Comparison of antioxidant activity of grape seed extract and fruits containing high β-carotene, vitamin C, and E. *Int. J. Food Prop.* **2013**, 16(3), 643-648.
26. Miller, J.N.; Miller, J.C. *Statistics and Chemometrics for Analytical Chemistry*, 6th ed., Pearson Education Limited: Essex, England; **2010**.
27. Tee, E.S.; Young, S.I.; Ho, S.K.; S. Siti Mizura, S. Determination of vitamin C in fresh fruits and vegetables using the dye-titration and microfluorometric methods. *Pertanika* **1988**, 11, 39-44.
28. Porto, I.S.A.; Neto, J.H.S.; dos Santos, L.O.; Gomes, A.A.; Ferreira, S.L.C. Determination of ascorbic acid in natural fruit juices using digital image colorimetry. *Microchem. J.* **2019**, 149, 104031.
29. Essodolom, P.; Chantal, B.E.; Mamatchi, M.; Kousanta, A. Effect of temperature on the degradation of ascorbic acid (vitamin C) contained in infant supplement flours during the preparation of porridges. *Int. J. Adv. Res.* **2020**, 8, 116-121.
30. Shrivastava, A.P.; Gupta, V.B. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists.* **2011**, 2, 21-25.