# Nutritional value and antioxidant properties of *Diospyros malabarica* (Desr.) Kostel., fruit from mid-hills of western Nepal

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## ABSTRACT

Fruit consumption has long been recognized for its beneficial impact on human health. Persimmon fruit *Diospyros malabarica* (Desr.) Kostel. is a popular wild edible fruit distributed in mid-hill regions of Western Nepal and is used in treatment of various ailments by local people. Although, persimmon is a popular fruit, its significant nutritive values and bioactive compounds are still unexplored in Nepal. This research on nutritional and phytochemical analysis of persimmon fruit pulp was carried out using standardized analytical methods (Association of Official Analytical Chemists, AOAC) and microplate spectrophotometry with ultraviolet (UV) detection. The results revealed that the fruit is rich in sugar (66.52±2.17 mg /g), fiber (103.95 mg/g), ash (107.44 mg/g) and total phenolic content (112±2.89 mg GAEs/g) and the mild supplier of antioxidants.

**Keywords:** Persimmon, *Diospyros malabarica*, Nutritional composition, Phytochemicals, Antioxidant activity, Nepal

## 1. INTRODUCTION

Fruits are supplements that provide health benefits and vital nutrition by holding nutritive values and secondary metabolites (Mohammed & Qoronfleh 2020). These components act beneficially to human physiology (Slavin & Lloyd 2012; Dreher 2018) as they provide defense against free radicals responsible for damaging lipids, proteins, and nucleic acids. Different vital organoleptic characteristics, including aroma, color, and nutritive values of the fruits, are dependent upon the secondary metabolites (Pott *et al.* 2019). Fruits are rich in antioxidants like polyphenols, selenium, vitamins C and E, lycopene, lutein, and other carotenoids, which are associated with several health benefits in human (Prior *et al.* 2003; Rangkadilok *et al.* 2007; Chalise *et al.* 2010). Those uncultivated

fruits (wild fruits) also possess abundant phytochemicals and hold good nutritive values. The wild fruits' antioxidant activities pursue a positive impact in maintaining balanced diets for a healthy body (Bvenura & Sivakumar 2017). Though wild fruits are exotic and underutilized, they contain various bioactive compounds and, provide health benefits such as free radical scavenging, anti-inflammatory, antioxidant, antimicrobial, and anticancer activities (Li *et al.* 2016).

Wild edible foods are popular in Nepal's rural areas where they are used as sources of fruits, juice, vegetables, and pickles (Bhattrai 2009). Wild edible foods play a vital role in food supplementation and nutrition in Nepal's local communities (Bajracharya 1980; Thapa 2014). In developing countries, including Nepal, wild edible plants contribute to earnings for indigenous people (Uprety 2012).

Persimmon fruit (*Diospyros malabarica* (Desr.) Kostel.) also known as 'Indian persimmon', 'Caqui', and 'Kaki' belongs to the largest genus of the Ebenaceae family, *Diospyros* spp. (Guo and Luo 2011; Díaz *et al.* 2020); capable to grow in different forest habitats ranging from lowland dry forest to rain forests (Yahia *et al.* 2011; Pino *et al.* 2014; García-Díaz *et al.* 2015; Xie *et al.* 2015; Rauf *et al.* 2017) that consists of more than 500 species (Rauf *et al.* 2017). *Diospyros* spp. is a multi-purposive plant (include fruits, timber, ornamental & medical uses) but is under-utilized (Rauf *et al.* 2017). The plant parts such as leaves, hardwood, roots, and fruits contain various pharmacological and nutraceutical properties (Pieroni *et al.* 2005; Martínez-Las Heras *et al.* 2015; Ding *et al.* 2017; Ma *et al.* 2018). However, occasionally, they can also be distributed in temperate regions in the form of deciduous and evergreen shrubs and trees (Xie *et al.* 2015; Rauf *et al.* 2017). *D. malabarica* is a flowering tree and medium-sized perennials; indigenous to the Indian sub-continent (Mondal *et al.* S). In Nepal, *D. malabarica*, commonly known as Teeju or Kaltu, can be found in very few areas like Palpa and Arghakanchi with altitude ranging from 500 m - 1500 m (Mahato *et al.* 2015; Paudel *et al.* 2017). The fruiting season for this plant is from February to June (Paudel *et al.* 2017).

In folklore medicine, *D. malabarica* fruits are useful in curing apthae, pharyngodynia, and digestive problems (Harborne 1994). The fruit pulp contains hexacosane, hexacosanol, ß-sitosterol, monohydroxy triterpene ketone, betulin, ß-D-glycoside of ß-sitosterol, betulinic acid, methyl ester acetate, methylester ß-D-glycoside of ß-sitosterol and these bioactive components contribute to curing the diseases (Kaushik *et al.* 2013). These plants also show medicinal effects against diabetes, diarrhea, dysentery, and other inflammatory diseases (Moniruzzaman *et al.* 2019; Shubhra 2019). Traditionally, the plant has also been used in the treatment of reproductive disorders in both male and female (Kaushik *et al.* 2013).

In Nepal, persimmon has often been consumed as fruits and is sometimes used by locals in disease treatment. However, in-depth analysis on nutritional values and phytochemical profile of persimmon found in this region remains to be explored. This present research focuses on quantitative analysis of specific macronutrients, active phytoconstituents, and antioxidant activity of persimmon *D. malabarica* from the Arghakhanchi district.

## 2. MATERIALS AND METHODS

### 2.1 Plants Samples

Ripened persimmon fruits were collected from Sandhikharka, Argakhanchi district in May 2018. Healthy fruits were carefully plugged from the bunch and air dried for about 20 days to remove moisture content. Then after, the samples were oven dried for a day at 37ºC. Seeds were separated from fruit pulp using forceps and needles. The dried pulp sample was finely powdered with the help of grinder and stored in airtight dry plastic vessel with proper labeling.

## 2.2 Nutrient Composition

### 2.2.1 *Protein Content Analysis*

Protein was estimated using a modified Bradford assay (Bradford 1976). In 200 mg of the dried powdered sample, 20 mL milli-Q water was added and incubated in hybridization shaking oven- MO-AOR (Innovative Life Science Tools, USA) at 50ºC in 100 RPM for 24 h. Then, the solution was filtered through Whatman no. 1 filter paper. The filtrate was used for determining protein content. The sample filtrate and a freshly prepared Bradford reagent were added in a 1:10 ratio into the microfuge tube. The test was assayed in triplicate. All the tubes were vortexed properly and incubated at room temperature (RT) for at least 5 min. Absorbance can increase over time; samples should incubate at RT for no more than 1 h (Stoscheck 1990). After that, 200 µL of each mixture was added into the individual well of 96 well plate, and their absorbance were measured at 595 nm against blank in a Multiskan Sky/ Microtitre spectrophotometer (ThermoFisher Scientific, USA) equipped with SkanIt software version 5.0. Bovine serum albumin (HiMedia Laboratories Private Ltd., India) was used to calculate the standard curve (10-500 µg/mL; $y=0.0012x+0.29$; $R^2 = 0.9941$) and the results were expressed in mg/g of sample.

### 2.2.2 *Carbohydrate Content*

This analysis was carried out by a colorimetric method using a modified anthrone reagent (Osborne & Voogt 1978). Pretreatment of 0.5 g of samples with 15 mL of 52% (v/v) $HClO_4$ and 10 mL of distilled water was performed and kept for 18 h in the dark (García-Herrera *et al.* 2020). After this period, samples were filtered, and the volume of the filtrate was diluted ten times. Then, 5 mL of 0.1% (w/v) anthrone solution in 70% (v/v) $H_2SO_4$ was added to 1 mL of sample extract. The mixture was kept in a boiling water bath for 12 min where the anthrone reaction with sugars yielded a green coloration. After that, 200 µL cooled mixture was added into the 96 well plate individually, and the absorbance was measured at 630 nm in the spectrophotometer. Glucose was used to calculate the standard curve (10-500 µg/mL; $y = 0.0023x + 0.0594$; $R^2 = 0.999$) and the results were expressed in mg/g of sample.

### 2.2.3 *Fat Content*

The dry sample (5 g) was weighed on a glazed paper and transferred into an extraction thimble. The thimble was introduced into Soxhlet extractor (SM Scientific Instruments, India) over a pad of cotton wool so that top of the thimble was well above the top of the siphon. A clean, dry, round bottom flask was weighed and fitted with the extractor. Then, 100 mL petroleum ether (HiMedia Laboratories Private Ltd., India) was poured along the side of the extractor until it begins to siphon off (Xiao *et al.* 2012). The equipment was assembled with the flask at 40ºC - 60ºC, and the extractor was connected to the condenser. The cool water circulation was started in the condenser, and the extraction was allowed for 6 h. The thimble was removed with the material from the extractor. The ether was evaporated at 40ºC to dryness using rotary evaporator-HS-2005 V-N (Hahnshin scientific co., South Korea). The receiver flask was dried in a hot air oven at 100ºC for 1 h, cooled and weighed. The result was expressed as mg/g of sample for fat content (Chew *et al.* 2011).

### 2.2.4 *Fiber Content*

Accurately, 2 gm of moisture-free and fat-free sample was weighed and transferred to the spoutless one-liter beaker. Then, 200 mL 1.25% $H_2SO_4$ was added in the beaker and placed on a hot plate; allowing to reflux for 30 min, time from onset of boiling. The content was shaken after every 5 min. The beaker was removed from the hot plate and filter through a muslin cloth using suction. The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker, and 200 mL of 1.25% NaOH solution was added and refluxed for 30 min. Again, filtration was performed, and the residue was washed with hot water until it was free from alkali. The total residue was transferred to a crucible and place in a hot air oven, allowed to dry to a constant weight at 80ºC - 110ºC, and weighed. The residue was ignited in a muffle furnace at 550ºC - 600ºC for 2-3 h, cooled, and weighed again. The loss of weight due to ignition was crude fiber's weight (Upadhyaya *et al.* 2017; Ogidi 2020).

### 2.2.5 *Ash Content*

Ash content was estimated using the dry ashing method (Chew *et al.* 2011). After weighing 10 g of sample in a silica crucible, the sample was incinerated in a cold muffle furnace set at 550ºC until whitish/greyish ash was obtained. The silica crucible was cooled, and the ash formed was weighed. The result was expressed as mg/g of sample for ash content.

## 2.3 Phytochemical Analysis

### 2.3.1 *Plant Extract Preparation*

Plant extract was prepared using modified Barros *et al.* (2007) protocol and used in determination of bioactive compounds in fruits. The dried and powdered persimmon fruit (10 g) was weighed separately, and 100 mL of methanol was added. The mixture was placed in a shaking incubator at 100 revolutions per min (RPM) at 37ºC for 24 h. The mixture was filtered through Whatman no. 1 filter paper and the filtrate was stored at 4ºC. Then, 100 mL methanol was added to the residue, and the mixture was placed again in a shaking incubator at 100 RPM, 37ºC for 24 h and filtered. The same step was repeated for two more times. All the filtrates from the 1st, 2nd, and 3rd filtration were mixed. The mixture was evaporated to dryness in a rotary evaporator at 40ºC. The dried methanolic extract was stored in a refrigerator until further analysis and it was re-dissolved in methanol when tests were performed.

### 2.3.2 *Stock Sample Preparation*

The methanolic plant extract (100 mg) was weighed, dissolved in 8 mL methanol, and the final volume was made to 10 mL to obtain a stock concentration of 10 mg/mL. Working solutions were prepared in methanol when needed.

### 2.3.3 *Phenolic Content*

Total phenolic content was estimated using a modified Folin-Ciocalteu assay (Singleton & Rossi 1965). To 50 µL of plant extract, 150 µL of Folin and Ciocalteu's phenol reagent (Merck Specialities Private Limited, Germany) was added. After 3 min, 150 µL of a saturated sodium carbonate (LOBA Chemie, India) solution was added to the mixture, and the volume was adjusted to 1500 µL with milli-Q water. The reaction was kept in the dark for 90 min. Then, 200 µL of the reaction was added to the 96 well plate, and absorbance was read at 725 nm in the spectrophotometer. Gallic acid (Fisher scientific, USA) was used to calculate the standard curve (25-500 µg/mL; $y = 0.0002x + 0.0446$; $R^2 = 0.9951$). The result was expressed as mg of gallic acid equivalents (GAEs) per g of extract.

### 2.3.4 *Flavonoid Content*

A modified aluminum chloride technique was used to calculate total flavonoid content. (Chang *et al.* 2002). The extract samples were diluted to 2.5 mg/mL and an aliquot (100 µL) of the diluted samples was placed in a 96 well ELISA plate, followed by adding 100 µL 0.1 M $AlCl_3$ solution (SD-fine-Chem Ltd, India). For control, methanol was taken in place of the sample while methanol was taken as blank. The same experiment was carried out with different quercetin concentration series (Sigma-Aldrich, Germany). Total flavonoid content of the plant sample was determined and expressed in milligrams of quercetin equivalent per gram of dry mass (mg QAEs/g) using the calibration curve obtained from a series of quercetin concentrations.

### 2.3.5 *Vitamin C Content*

To determine vitamin C content, the modified Klein and Perry (1982) method was used. For this, 50 mg dry methanolic extract of *D. malabarica* was extracted in 5 mL of 1% meta-phosphoric acid (HiMedia Laboratories Private Ltd., India) for 45 min at room temperature. The solution was filtered through Whatman No. 4 filter paper. To 100 µL of each sample, 900 µL of 2,6-dichlorophenolindophenol (HiMedia Laboratories Private Ltd., India) was added and mixed properly. 200 µL of reaction was added to 96 well plates, and the absorbance was measured within 30 min at 515 nm against a blank in the spectrophotometer. L-ascorbic acid (CDH Laboratory reagents, India) was used to calculate the standard curve (25-100 µg/mL; $y = -0.0019x + 2.293$; $R^2 = 0.9986$). The result was expressed as mg of ascorbic acid/g of extract.

### 2.3.6 *β-carotene and Lycopene Content*

To determine β-carotene and lycopene content, modified Nagata and Yamashita (1992) were used. After weighing 100 mg dried methanolic extract, 10 mL of acetone-hexane mixture (4:6) was added for 1 min. The mixtures were shaken vigorously and filtered through Whatman no. 4 filter paper. After that, 200 µL of the sample was added to the ELISA plate and absorbance was measured at 453, 505, and 663 nm, respectively in the spectrophotometer. Contents of β-carotene and lycopene were calculated according to the following equations: Lycopene (mg/ 100 mL) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$ and, β-carotene (mg/ 100 mL) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$.

The results were expressed as µg of lycopene/g and µg of carotenoid/g respectively.

## 2.4 Diphenyl picrylhydrazyl (DPPH) Radical-Scavenging Activity

DPPH Radical-Scavenging activity was determined with the modified Blois method (1958). Various *D. malabarica* extracts (100 µL) concentrations were mixed with 900 µL of methanolic solution containing DPPH (Sigma-Aldrich, Germany) radicals ($6\times10^{-5}$ mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). After that, 200 µL of each reaction mixture was added to the ELISA plate reader, and the reduction of the DPPH radical was determined by measuring the absorption at 517 nm in the spectrophotometer. The RSA was calculated as a percentage of DPPH discoloration using the equation % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$. Where $A_S$ is the absorbance of the solution when the sample extract has been added at a particular level and $A_{DPPH}$ is the absorbance of the DPPH solution. The extract concentration providing 50% of RSA ($EC_{50}$) was calculated from the RSA percentage graph against extract concentration. Ascorbic acid was used as standards.

## 2.5 Statistical Analysis

All the assays were conducted in triplicate except fat, fiber and ash contents (n=1). The results were expressed as mean values and standard deviation (SD). The results were analyzed using MS excel for Microsoft 365 and SPSS version 22.

## 3. RESULTS AND DISCUSSION

### 3.1 Nutritional Analysis

We are first to report the nutritional value in persimmon fruit *D. malabarica* in Nepal. Fig. 1 shows the result of nutritional content (protein, carbohydrate, fat, fiber and ash) of the fruit.

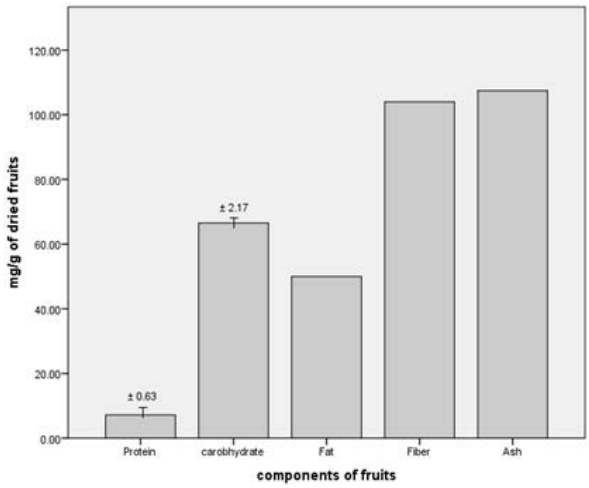


Fig 1. Nutritional analysis of persimmon fruit

### 3.1.1 *Protein Content*

Persimmon fruit showed 7.17±0.63 mg/g of protein content. The recent value is lower than the previous reports on protein of *D. malabarica*; 13.6 mg/g and 40.77 mg/g (Sajib *et al.* 2014; Sarmah *et al.* 2013). Nutritional composition of plants is affected by various factors like soil, weather and climate as well as fertilizer (Hornick 1992). Plant foods are the great source of organic and mineral nutrients but a minor source of protein for human (DellaPenna & Grusak 1999).

### 3.1.2 *Carbohydrate Content*

Fruit is a good source of carbohydrate (DellaPenna and Grusak 1999). The carbohydrate content in persimmon fruit was 66.52±2.17 mg/g. Higher carbohydrate levels were reported in *D. malabarica* 85.6 mg/g and 203.4 mg/g (Sajib *et al.* 2014; Sarmah *et al.* 2013). This may be attributed to the ripening stage of the fruits as well as soil factors, climatic factors and geographical variations (Haque *et al.* 2009; Hornick 1992). Thus, persimmon fruit is a good source of carbohydrate.

### 3.1.3 *Fat Content*

Fatty acids are one of important source of energy supply in human. Plants are good source of fatty acids like linoleic and linolenic acid and both are essential for human (DellaPenna & Grusak 1999). Fatty acids are crucial for the growing fetus and are also essential for the brain's functioning (Stubbs *et al.* 2018). Persimmon fruit contained 49.96 mg/g of fat content. Sajib *et al.* (2014) reported 1.7±0.6 mg/g fat from fresh *D. malabarica* which was exceptionally lower than dried persimmon fruit used in this study. Fat contents in fruit depend on type of sample used. Reports on *Diospyros kaki* showed high fat content in dried fruit (5.9 mg/g) than in raw fruits (1.9 mg/g) (Pachisia 2020). Fat content also depends in different stages of fruit (Ayaz & Kadioglu 1999). Viswanathan *et al.* (2002) reports the oil from *D. malabarica* fruit contains *trans-α-methyl* isoeugenol; an oviposition stimulant and, ß-asarone; a known tranquilizer and anticancer agent.

### 3.1.4 *Fiber Content*

Total dietary fiber is a combination of water-insoluble and water-soluble fiber (Díaz *et al.* 2020). The high content of dietary fiber is associated with reduction of cardiovascular risk factors and diabetes mellitus (Van Duyn & Pivonka 2000; Díaz *et al.* 2020). The total fiber in persimmon fruit was in high value (103.95 mg/g). A report in *Diospyros kaki* showed 145 mg/g fiber content in the fruit (Pachisia 2020). High fiber contents were also seen in other fruits like *Mordii whytii* (118 mg/g) (Adepoju 2009), *Ziziphus abyssinica* (9.78%) and, *Vitellaria paradoxa* (15.9%) (Loki & Ndyomugyenyi 2016). The fiber content in persimmon fruits is higher than more popular fruits like apple, orange, pear, peach and banana (Díaz *et al.* 2020), thus, it makes *D. malabarica* fruit an excellent fiber source for human.

### 3.1.5 *Ash Content*

Ash is produced when organic matter is burned off, and the inorganic material remained (Tee *et al.* 1996). Persimmon fruit showed 107.44 mg/g of ash content which is 10.74% of its dried weight; suggesting presence of high minerals. Sajib *et al.* (2014) reported lower value of ash content (0.88±0.08 %) in *D. malabarica*. Celik and Ercisli (2008) reported 0.44% of ash content in *Diospyros kaki*. Ash content higher than 17% were found in leafy vegetables like spinach and radish. The value of ash content is highly dependent on type of drying methods (Sonkamble *et al.* 2015). Ash content measures the total amount of macro minerals like Na, K, Ca and Cl and micro minerals like Fe, Cu, Mn and Zn in plant (Sonkamble *et al.* 2015; Díaz *et al.* 2020). The mineral content in plant depends in soil composition and availability of minerals in the plant environment (DellaPenna & Grusak 1999). Minerals play important role in human health. Magnesium prevents atherosclerosis whereas potassium lowers the risk of hypertension. Minerals have significant role in enzymatic reaction in human body (Díaz *et al.* 2020). Further analysis of ash with atomic absorption spectroscopy can bring insight on mineral composition of persimmon fruit.

## 3.2 Phytochemical Analysis

Phytochemicals are chemical compound found in plants which show beneficial effect on human health (DellaPenna & Grusak 1999). Phytochemical analysis of *D. malabarica* fruit has not been analyzed in Nepal. This is the first study to report phytochemical analysis in the fruit. Table 1. shows phytochemicals (phenol, flavonoid, vitamin C, β-carotene and lycopene) present in persimmon fruit.

Table 1. Phenols, flavonoids, ascorbic acid, β-carotene and lycopene contents (mean ± SD) in methanolic extract.

| Sample | Phenol (mg GAE/g) | Flavonoid (mg QAE/g) | Vitamin C (mg/g) | β-carotene (µg/g) | Lycopene (µg/g) |
|---|---|---|---|---|---|
| *D. malabarica* | 112±2.89 | 6.97±0.07 | ND | 54.30±1.69 | 141.141±2.33 |

### 3.2.1 *Phenol and Flavonoid*

The total phenolic content in persimmon fruit was significantly high i.e., 112±2.89 mg of GAEs/g of extract. High phenol content (223.5±0.26 mg GAE/g) was reported on fruit collected from Assam (Sarmah *et al.* 2013). Reports on low phenolic value ranged from 0.434 mg GAE/g to 12.87 mg GAE/g (Rehman *et al.* 2020; Moniruzzaman *et al.* 2019).

The total flavonoid content of persimmon fruit was 6.97±0.07 mg QAEs/g of extract. Rehman *et al.* (2020) reported lower value of TFC (64.7±7 mg of GAEs/100g). The differences in values of TPC and TFC may be associated to various intrinsic and extrinsic factors like soil and crop conditions, climate and weather conditions and also on stage of fruit during sampling (Díaz *et al.* 2020). The phenol and flavonoid play an important role in preventing diseases as they adsorb and neutralizes free radicals (Pandey & Rizvi 2009). Free radicals like reactive oxygen species and reactive nitrogen species are a byproduct resulting from cellular metabolism which are crucial to the body (Ansari *et al.* 1997). When their generation is beyond normal, they may induce human chronic diseases, including atherosclerosis, cancer, diabetes mellitus, cataract, rheumatoid arthritis, auto-immune disorder, and Parkinson's disease (Halliwell *et al.*1990; Gih-Mahapel *et al.* 2014).

### 3.2.2 *Vitamin C*

Vitamin C is an antioxidant which plays a crucial role in metabolism and absorption of iron as well as collagen (Chambial *et al.* 2013). Dietary antioxidants are non-toxic natural compounds that can control human diseases (Lobo *et al.* 2010). Deficiency of vitamin C can cause disease like anemia and scurvy (Chambial *et al.* 2013). In our study, vitamin C was not detected in persimmon fruit. Shubhra (2019) reported insignificant vitamin C in aqueous flesh extract of *D. malabarica*. Some studies found very low level of vitamin C; 55.57 mg/100g (Sarmah *et al.* 2013) and 14.25 mg/100g (Sajib *et al.* 2014). Thus, persimmon fruit is not a good source of vitamin C.

### 3.2.3 *β-carotene and Lycopene*

Carotenoids are fat-soluble pigments which has many health benefits in human. β-carotene is one of the major sources of vitamin A whereas lycopene is an important antioxidant (Díaz *et al.* 2020). β-carotene and lycopene level in persimmon fruits were 54.30±1.69 µg/g and 141.141±2.33 µg/g. The recent values were higher than previous studies which reported broad range of β-carotene (10.07-374 µg/100g) and lycopene (17.51–53.50 µg/100g) level in *D. kaki* (Díaz *et al.* 2020; Pachisia 2020). The level of β-carotene and lycopene varied widely on batch and season of fruit collected (Díaz *et al.* 2020). Various environmental factors like soil, season, climate and fruit batch directly affect the level of β-carotene in fruits (Díaz *et al.* 2020).

Table 2. DPPH radical scavenging activity (mean ± SD %) of standard ascorbic acid

| | *Concentration of methanolic extract (µg/mL)* | | | | | |
|---|---|---|---|---|---|---|
| **Ascorbic acid** | **5** | **10** | **25** | **50** | **100** | **250** |
| **% RSA** | 16.87±2.68 | 25.57±2.89 | 53.11±0.90 | 83.54±0.0 | 84.47±0.18 | 84.47±0.0 |

Table 3.DPPH radical scavenging activity (mean ± SD %) of persimmon fruit

| | *Concentration of methanolic extract (µg/mL)* | | | | | |
|---|---|---|---|---|---|---|
| ***D. malabarica*** | **100** | **250** | **500** | **750** | **1000** | **2000** |
| **% RSA** | - | - | 17.39±1.61 | 32.30±1.29 | 44.10±2.25 | 75.47±0.78 |

### 3.2.4 *DPPH Radical Scavenging Activity*

DPPH radical scavenging activity is method to evaluate the antioxidant potential of a chemical compound (Kedare & Singh 2011). Table 2 and 3 shows the DPPH radical scavenging activity of standard ascorbic acid and persimmon fruit extract, respectively. Ascorbic acid showed strong radical scavenging activity compared to persimmon fruit extract. Fig. 2 shows *in vitro* antioxidant activity of ascorbic acid and persimmon fruit. The $EC_{50}$ of persimmon fruit (1122.02±115.9 µg/mL) was much higher than standard ascorbic acid (22.91±0.71 µg/mL). Ascorbic acid's low $EC_{50}$ value facilitates in combating free radicals in lower concentration and makes it as an excellent antioxidant. On the other hand, persimmon fruit's high $EC_{50}$ suggests its weak antioxidant nature.

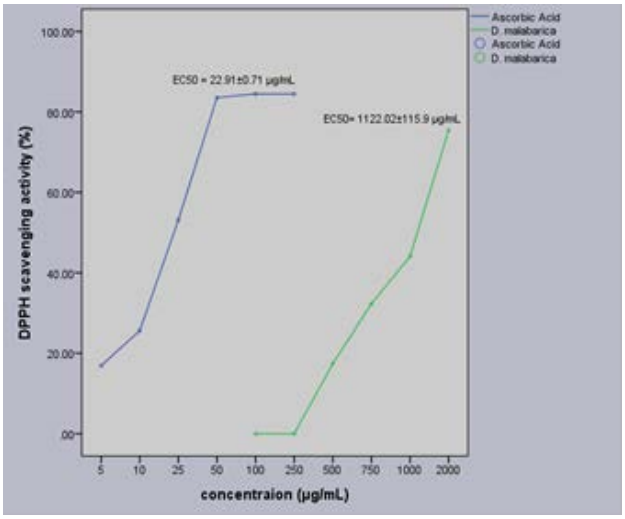


Fig 2. *In vitro* antioxidant activity of ascorbic acid

Rice-Evans *et al.* (1996) found that flavonoids like quercetin and epigallocatechin were good antioxidant than phenolic acids like gallic acid. Pereira *et al.* (2018) reported strong antioxidant activities of calabura fruits even in lower amount of flavonoids like catechin, gallocatechin, epigallocatechin, naringenin and quercetin. Blueberries with high levels of flavonoids like proanthocyanidins and anthocyanidins also showed high antioxidant activity (Huang *et al.* 2011). In this work, the low flavonoid value in persimmon fruit can contribute to its low antioxidant capacity. A complete profile analysis of phenol and flavonoid could be suggested to co-relate them with low antioxidant activity of persimmon fruit.

## 4. CONCLUSION

Persimmon fruit, an under-utilized plant in Nepal contains nutrients such as protein, carbohydrate, fat, fiber and mineral as well as phytochemicals like phenol, flavonoid and carotenoids. The fruit is a good source of fat, fiber, carbohydrate, minerals, β-carotene and lycopene. Although it contains high-level of phenol, its low flavonoid value makes it a mild antioxidant; antioxidant activity depends mostly on flavonoid. The utilization of *D. malabarica* fruit in food as well as pharmaceutical industry may be a good approach to enhance human health.

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