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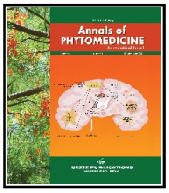

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Effect of temperature and time on the antioxidant activity of fresh and dried persimmon pulp and seed extracts

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Abstract

The "antioxidant properties of wild Persimmon fruit (*Diospyros virginiana*) available in Kashmir valley" were carried out during the year 2021. The estimation of antioxidant properties of different extracts of fresh and dried persimmon pulp and seeds using a common solvent under different conventional and microwave-assisted extraction conditions were carried out. The extracts were analysed for antioxidant properties using four different estimation methods α, α-diphenyl-β-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The fresh pulp sample extract at 60°C presented the highest antioxidant capacity in terms of DPPH radical scavenging activity (93.23%) in comparison to other samples in conventional extraction method. While in microwave extraction method, fresh pulp sample extract at 90 sec exposure time showed the highest (95.00%) DPPH radical scavenging activity. The highest antioxidant activity in terms of ABTS is shown by fresh pulp sample extract (99.83%) at 50°C in conventional extraction method. While the fresh pulp sample extract at 60 sec exposure time showed the highest (99.66%) ABTS scavenging activity in microwave extraction method, respectively. Fresh seed sample extract at 60°C showed the highest antioxidant capacity (1209.21 µMTE/g) in terms of FRAP assay in conventional extraction method. While in microwave extraction method, fresh seed sample extract at 90 sec showed the highest antioxidant capacity (1259.9 µMTE/g.)

1. Introduction

Persimmon is fleshy fibrous tropical fruit belonging to Ebenaceae family. It is native to Japan, China, Korea, Burma and Nepal (Choudhary *et al*., 2022). It is a deciduous tree and flowers from July to August and seeds ripen in November (Sonal *et al*., 2022). In color, the ripe fruits of persimmon ranges from light yellow-orange to dark red-orange depending on the species and variety and in shape. The varieties may be spherical, acorn or pumpkin shaped (Petersen and Martin, 2007). It is frequently grown in warm climates around the world, such as China, Korea, Japan, Brazil, Turkey, and Italy (Itamura *et al*., 2005; Yokozawa *et al*., 2007). In 2017, the global production of persimmon reached over 7.9 million tonnes (FAO, 2018). According to FAOSTAT Database, 2019, China is producing 3,092,000 tonnes, Spain 404,131tonnes, Korea 298,382 tonnes, Japan 224,900 tonnes, Brazil 182,185 tonnes, Azerbaijan 147,219 tonnes, Uzbekistan 88,233 tonnes, Italy 49,675 tonnes, Israel 29,000 tonnes, and Iran 24,257 tonnes (Giordani, 2022). The persimmon market in India is limited. However, persimmons are grown in a number of India's cooler regions, including as Jammu and Kashmir, Himachal Pradesh, and the highlands of Uttar Pradesh. In India, the agro-climatic conditions of northern states such as Himachal Pradesh, Jammu and Kashmir, Uttrakhand and parts of Nilgiri hills of south are suitable for cultivation of persimmon. In Himachal Pradesh, district Mandi is the highest producer of persimmon, followed by Kullu and Shimla. The area under cultivation is reported to be 421 hectare with the production of 943 metric tons (Gautam *et al*., 2020). The nutritional value of persimmon is well known (Nafeesa *et al*., 2021) containing 80% water, 0.58% protein, 0.19% total fats, 16.60% total carbs, certain minerals (magnesium, iron, zinc, copper, manganese, *etc*.), and up to 1.48 g and 7.50 mg total dietary fibre and ascorbic acid, respectively (Ozen *et al*., 2004; Ercisli *et al*., 2007). Recent research shown that persimmon also contributes to the availability of calcium and potassium. Persimmons have higher sugar content (12.5 g/100 g) than other widely consumed fruits like apple, peach, pear, and orange. Sucrose and its monomers (glucose and fructose) are the most prevalent sugars (Altuntas *et al*., 2011). Proanthocyanidins (Jung *et al*., 2005; Suzuki *et al*., 2005), flavonoid oligomers, tannins, phenolic acids, catechin (Lee *et al*., 2012; Jo *et al*., 2003), carotenoids, and tannins are some of the predominant phytochemicals found in persimmon leaves and fruits (Yokozawa *et al*., 2007). The

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persimmon's rich phytochemistry has sparked new lines of inquiry into diet-based treatments for a variety of diseases.

A growing number of people are interested in natural antioxidants that are safer and come from plants, especially those that can be utilised in foods and medicines to reduce oxidative damage (Gulcin *et al.*, 2006). Particularly whenever free radical generation is involved, more focus has been placed on the investigation of the antioxidative and antilipid peroxidation activity of natural dietary antioxidants and their protective benefits against drug-induced toxicities (El-Beshbishy *et al.*, 2009). The most widely used synthetic antioxidants in food are butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), both of which have excellent antioxidant properties. However, due to their instability or possible role as carcinogenesis promoters, their use in food products has been declining (Msaada *et al.*, 2013). Additionally, natural antioxidants have the power to enhance the quality and consistency of food as well as function as nutraceuticals to stop free radical chain reactions in biological systems, which may provide consumers extra health benefits (Zhao *et al.*, 2014). Persimmons being high in moisture content are mainly preserved by the process of drying in order to extend the shelf-life and make the fruit available in off-season. Persimmons when dried are classified in two groups: Semi dried with moisture content of 50% and dried persimmons with moisture content of 35%. Production of dried persimmons thus makes commercialization and exportation time to be enhanced (Vilhena *et al.*, 2020). Keeping in view, the health benefits of natural antioxidants, traditional use of persimmon fruit to cure various ailments, the present study was carried on persimmon fruit with the objective to study the antioxidant potential of wild persimmon effect of temperature and time on antioxidant activity of fresh and dried persimmon pulp and seed extracts.

2. Materials and Methods

The present investigation was conducted at the Department of Food Science and Technology, SKUAST(K).

2.1 Raw materials

Fresh persimmons were collected from a local farm in Nishat area, Srinagar, Jammu and Kashmir and brought to the Food Processing and Training Centre (FPTC) laboratory and stored under room temperature. The fruits were washed properly to remove any possible contaminations. In order to obtain pulp from the fruit, cold pulping method was used. After washing, the calyx were removed manually with the help of stainless-steel knife and cut into halves. The seeds and the peel were also removed manually. The fruits after cutting into small pieces were converted into pulp by grinding in a mixer-cum grinder (Wonderchef Model No: 63152172). For antioxidant profiling fresh and dried persimmons seeds and pulp were used. The pulp and seeds of the group intended for drying were heated to different time and temperature combinations (45, 50, 55, 60, 65°C for 12 h, respectively, while the pulp and seeds intended for fresh usage were cryogenically frozen using liquid nitrogen, packaged in high density polyethylene (HDPE) pouches, and stored in a deep freezer (–30°C) until further use.

The dried persimmon fruit parts were ground in a grinder to obtain powder for further analysis which was packed in high density polyethylene (HDPE) pouches and stored at ambient temperature (Temp. 4.32 to 15.73°C, RH 66.00 to 80.00%) till further use.

2.2 Antioxidant assay

2.2.1 Methods of extraction

Two extraction methods with different variables; temperature and time and with a common solvent were followed for potential extraction of antioxidants. The first method used for extraction was the traditional method (Xu, *et al.*, 2017) and the other method used was the microwave-assisted extraction (Li *et al.*, 2017).

2.2.2 Assay

Antioxidant assay in terms of total phenolic content, DPPH, ABTS, FRAP and CUPRAC, were conducted using standard procedures:

2.2.2.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (Rajurkar and Hande, 2011)

With some changes, the Brand-Williams *et al.* (1995) technique for the DPPH assay was used. 24 mg of DPPH was combined with 100 ml of methanol to create the stock solution, which was then kept at 20°C until required. Using a spectrophotometer, 10 ml of stock solution and 45 ml of methanol were combined to create the working solution, which had an absorbance of 1.170 units at 515 nm. 150 µl of fruit extracts were combined with 2850 µl of the DPPH solution and left to react for 24 h in the dark. Next, the absorbance at 515 nm was measured. Using the following formula, the antioxidant activity was reported as the scavenging percentage.:

$$\% \text{ Inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

2.2.2.2 ABTS (2,2-azinobis (3-Ethylbenzothiazoline-6-sulfonic acid) (Rajurkar and Hande, 2011)

The stock solutions included 2.6 mM potassium persulfate solution and 7.4 mM ABTS. + solution. The two stock solutions were then combined in equal parts to create the working solution, which was then left to react for 12 h at room temperature and in the dark. The solution was then diluted to produce an absorbance of 1.10.02 units at 734 nm using the spectrophotometer by combining 1 ml of ABTS. + solution with 60 ml of methanol. For each test, a brand-new ABTS solution was created. Sample extracts (150 µl) were mixed with 2850 µl of the ABTS solution and left to react for two hours in the dark before the absorbance at 734 nm was measured using a spectrophotometer. Results were determined using the following formula and expressed as a scavenging percentage:

$$\text{Scavenging (\%)} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

2.2.2.3 FRAP (Ferric-reducing antioxidant power) assay (Rajurkar and Hande, 2011)

The stock solutions contained 20 mM $FeCl_3.6H_2O$ solution, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution, and 300 mM acetate buffer (3.1g $C_2H_3NaO_2.\ 3H_2O$ and 16 ml $C_2H_4O_2$) at pH 3.6. By combining 25 ml of acetate buffer, 2.5 ml of TPTZ solution, and 2.5 ml of $FeCl_3.6H_2O$ solution, the fresh working solution was created. It was then warmed at at 37°C before use. Sample extracts (150 l) were combined with 2850 µl of the FRAP solution and left to react for 30 min in the dark. The coloured substance (ferrous tripyridyltriazine complex) was then read at 593 nm.The standard

curve was linear between 25 and 800 µM Trolox. Results were expressed in µM TE/g fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

2.2.2.4 CUPRAC (Cupric ion reducing antioxidant capacity) (Rajurkar and Hande, 2011)

To a test tube was added 1 ml each of copper chloride (Cu (II)), neocuproine (Nc) and ammonium acetate buffer (NH_4Ac) solutions. Antioxidant sample (or standard) solution (x ml) and H_2O (1.1 - x) ml were added to the initial mixture so as to make the final volume 4.1 ml. The tubes were stoppered and after 2 h, the absorbance at 450 nm (A_{450}) was recorded against a reagent blank, developed on the same way but the extract was replaced with methanol. A standard curve was created with Trolox. The results were expressed in µM Trolox (TE) per g DW.

2.3 Statistical analysis

Experimental data were determined in triplicates and reported as average of the replications. Statistical analysis was carried using commercial Minitab. Results were subjected to two-way analysis of variance to determine the significance of differences ($p \leq 0.05$).

3. Results

3.1 Antioxidant activity of different sample extracts of persimmon fruit

The antioxidant capacity of four different sample extracts (fresh pulp, fresh seed, dried pulp, dried seed) of persimmon fruit using a common solvent (acetonitrile) was evaluated by 4 assays; 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and cupric ion reducing antioxidant capacity (CUPRAC). Each antioxidant assay possesses its own unique mechanism to evaluate the antioxidant activity in different samples of persimmon fruit.

3.1.1 DPPH radical scavenging activity by using conventional and microwave assisted solvent extraction methods

In conventional solvent extraction method (Table 1), the fresh pulp sample extract of persimmon fruit showed significantly higher mean DPPH radical scavenging activity (74.72%), followed by fresh seed sample extract (40.49%), dried pulp sample extract (37.11%) and the dried seed sample extract which showed the least scavenging activity of (13.22%).

Table 1: Effect of extraction temperature on DPPH (%) radical scavenging activity of persimmon fruit sample extracts using conventional extraction method

Temp. (°C)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
40	$60.63^{aA} \pm 0.01$	$32.12^{aB} \pm 0.01$	**46.37**	$31.81^{aA} \pm 0.02$	$11.03^{aB} \pm 0.02$	**21.42**
50	$70.30^{bA} \pm 0.17$	$40.45^{bB} \pm 0.02$	**55.37**	$37.15^{bA} \pm 0.02$	$12.51^{bB} \pm 0.01$	**24.83**
60	$93.23^{cA} \pm 0.02$	$48.92^{cB} \pm 0.02$	**71.07**	$42.38^{cA} \pm 0.01$	$16.12^{cB} \pm 0.01$	**29.25**
Mean	**74.72**	**40.49**	**57.60**	**37.11**	**13.22**	**25.16**

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of temperature, Uppercase alphabetical letters represent difference between pulp and seed.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of four different sample extracts of persimmon fruit using microwave assisted solvent extraction is given in Table 2.

Table 2: Effect of extraction time on DPPH (%) radical scavenging activity of persimmon fruit sample extracts using microwave-assisted extraction method

Time (sec)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
30	$65.00^{aA} \pm 0.20$	$38.83^{aB} \pm 0.035$	**51.91**	$37.47^{aA} \pm 0.02$	$12.19^{aB} \pm 0.03$	**24.83**
60	$71.00^{bA} \pm 0.005$	$47.33^{bB} \pm 0.030$	**59.16**	$42.67^{bA} \pm 0.011$	$30.67^{bB} \pm 0.02$	**36.67**
90	$95.00^{cA} \pm 0.00$	$72.63^{cB} \pm 0.01$	**83.81**	$59.26^{cA} \pm 0.02$	$49.53^{cB} \pm 0.01$	**54.39**
Mean	**77.00**	**52.93**	**64.96**	**46.46**	**30.79**	**38.63**

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of time, Uppercase alphabetical letters represent difference between pulp and seed.

3.1.2 ABTS scavenging activity by using conventional and microwave-assisted solvent extraction methods

The values obtained for ABTS assay for conventional and microwave-assisted solvent extraction using a common solvent are given in Tables 3 and 4.

Table 3: Effect of extraction temperature on ABTS (%) scavenging activity of persimmon fruit sample extracts using conventional extraction method

Temp. (°C)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
40	$99.43^{aA} \pm 0.00$	$97.51^{aB} \pm 0.09$	**98.47**	$93.31^{aA} \pm 0.12$	$91.82^{aB} \pm 0.08$	**92.56**
50	$99.83^{bA} \pm 0.03$	$97.85^{bB} \pm 0.04$	**98.84**	$97.71^{bA} \pm 0.09$	$92.55^{bB} \pm 0.13$	**95.13**
60	$98.31^{cA} \pm 0.01$	$97.23^{cB} \pm 0.01$	**97.77**	$94.41^{cA} \pm 0.09$	$87.81^{cB} \pm 0.11$	**91.11**
Mean	**99.19**	**97.53**	**98.36**	**95.14**	**90.72**	**92.93**

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of temperature, Uppercase alphabetical letters represent difference between pulp and seed.

Table 4 represented the data of ABTS scavenging activity of microwave-assisted extraction method in persimmon fruit extracts (pulp and seed). Among all the four persimmon fruit extracts, it was found that fresh pulp sample extract showed the highest ABTS scavenging activity of 99.66%.

Table 4: Effect of extraction time on ABTS (%) scavenging activity of persimmon fruit sample extracts using microwave-assisted extraction method

Time (sec)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
30	$99.60^{aA} \pm 0.10$	$97.80^{aB} \pm 0.10$	**98.70**	$96.70^{aA} \pm 0.11$	$91.97^{aB} \pm 0.01$	**94.33**
60	$99.66^{bA} \pm 0.03$	$99.30^{bB} \pm 0.15$	**99.48**	$97.40^{bA} \pm 0.17$	$93.86^{bB} \pm 0.00$	**95.63**
90	$99.26^{cA} \pm 0.05$	$98.20^{cB} \pm 0.11$	**98.73**	$95.56^{cA} \pm 0.01$	$89.13^{cB} \pm 0.01$	**92.34**
Mean	**99.50**	**98.43**	**98.97**	**96.55**	**91.65**	**94.10**

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of time, Uppercase alphabetical letters represent difference between pulp and seed.

3.1.3 FRAP (ferric-reducing antioxidant power) analysis by using conventional and microwave-assisted solvent extraction methods

Reducing power (RP), which is correlated with some substances' antioxidant capacity (Jayaprakash *et al.*, 2001), may be a useful indicator of possible antioxidant activity (Meir *et al.*, 1995). By evaluating the reduction of ferricyanide complexes, Fe^{3+} form to their ferrous (Fe^{2+}) form, reducing power was ascertained. The antioxidant activities evaluated by the FRAP (ferric reducing antioxidant power) assay of four different sample extracts of persimmon fruit using conventional and microwave assisted solvent extraction are presented in Tables 5 and 6, respectively.

As for the conventional extraction method, the antioxidant activity by FRAP method of different sample extracts of persimmon fruit are presented in Table 5

Table 5: Effect of extraction temperature on FRAP (µMTE/g) of persimmon fruit sample extracts using conventional extraction method

Temp. (°C)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
40	$623.6^{aA} \pm 0.01$	$1017.43^{aB} \pm 0.03$	**820.51**	$498.66^{aA} \pm 0.15$	$949.66^{aB} \pm 0.25$	**724.16**
50	$628.16^{bA} \pm 0.11$	$1137.5^{bB} \pm 0.02$	**882.83**	$518.13^{bA} \pm 0.15$	$1049.66^{bB} \pm 0.25$	**783.89**
60	$634.68^{cA} \pm 0.05$	$1209.21^{cB} \pm 0.01$	**921.94**	$523.62^{cA} \pm 0.02$	$1185.00^{cB} \pm 0.00$	**854.31**
Mean	**628.81**	**1121.38**	**875.09**	**513.47**	**1061.44**	**787.45**

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of temperature, Uppercase alphabetical letters represent difference between pulp and seed.

The FRAP activity of microwave-assisted persimmon pulp and seed extracts is shown in Table 6.

Table 6: Effect of extraction time on FRAP (µMTE/g) antioxidant activity of persimmon fruit sample extracts using microwave-assisted extraction method

Time (sec)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
30	$744.0^{aA} \pm 0.26$	$1058.3^{aB} \pm 0.2$	901.15	$703.1^{aA} \pm 0.1$	$1008.7^{aB} \pm 0.2$	855.9
60	$1161.2^{bA} \pm 0.15$	$1169.8^{bB} \pm 0.17$	1165.5	$1157.4^{bA} \pm 0.05$	$1119.9^{bB} \pm 0.1$	1138.65
90	$1253.93^{cA} \pm 0.04$	$1259.9^{cB} \pm 0.1$	1256.91	$1160.0^{cA} \pm 0.1$	$1191.13^{cB} \pm 0.15$	1175.56
Mean	1053.04	1162.66	1107.85	1006.83	1106.57	1056.70

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of time, Uppercase alphabetical letters represent difference between pulp and seed.

3.1.4 CUPRAC (cupric reducing antioxidant power) analysis by using conventional and microwave-assisted solvent extraction methods

The CUPRAC activity of persimmon pulp and seed extracts by conventional extraction are shown in Table 7.

Table 7: Effect of extraction temperature on CUPRAC (µMTE/g) antioxidant activity of persimmon fruit sample extracts using conventional extraction method

Time (sec)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
30	$455.06^{aA} \pm 0.05$	$863.70^{aB} \pm 0.10$	**659.38**	$426.10^{aA} \pm 0.10$	$813.86^{aB} \pm 0.05$	**619.98**
60	$489.13^{bA} \pm 0.23$	$1048.73^{bB} \pm 0.11$	**768.93**	$470.10^{bA} \pm 0.17$	$890.73^{bB} \pm 0.05$	**680.41**
90	$494.12^{cA} \pm 0.01$	$1112.46^{cB} \pm 0.02$	**803.29**	$476.21^{cA} \pm 0.01$	$966.13^{cB} \pm 0.15$	**721.17**
Mean	**479.43**	**1008.29**	**743.86**	**457.47**	**890.24**	**673.85**

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of time, Uppercase alphabetical letters represent difference between pulp and seed.

As for the CUPRAC of microwave extracts of different samples of persimmon fruit presented in Table 8, the trend is similar to that of conventional extraction.

Table 8: Effect of extraction time on CUPRAC (µMTE/g) antioxidant activity of persimmon fruit sample extracts using microwave-extraction method

Time (sec)	Fresh			Dried		
	Pulp	Seed	Mean	Pulp	Seed	Mean
30	$568.1^{aA} \pm 0.1$	$872.68^{aB} \pm 0.01$	**720.39**	$510.10^{aA} \pm 0.1$	$823.5^{aB} \pm 0.1$	**666.8**
60	$880.56^{bA} \pm 0.15$	$1084.16^{bB} \pm 0.02$	**982.36**	$868.66^{bA} \pm 0.01$	$956.7^{bB} \pm 0.15$	**912.68**
90	$1075.5^{cA} \pm 0.1$	$1151.13^{cB} \pm 0.02$	**1113.31**	$1007.30^{cA} \pm 0.2$	$1110.5^{cB} \pm 0.06$	**1058.9**
Mean	**841.38**	**1035.99**	**938.68**	**795.35**	**963.56**	**879.46**

Values with different superscripts differ significantly ($p \leq 0.05$), Lowercase alphabetical letters represent effect of time, Uppercase alphabetical letters represent difference between pulp and seed.

4. Discussion

Fresh persimmon pulp and seed extracts were found to have greater DPPH radical scavenging capacity than their dried counterparts. This drop in total phenolic content during dehydration may be attributed to polyphenols interacting with other chemicals or changing their chemical structure, which cannot be removed and measured using the methods currently in use. Gorinstein *et al.* (2006) reported similar findings, showing that fresh persimmons had much higher total antioxidant activity than dried persimmons as measured

by the DPPH and ABTS assays. It is also evident from the Table 1 that the mean DPPH value of pulp extracts was more as compared to seed extracts. This could be due to presence of major phenolic acids in the persimmon pulp which are responsible for antioxidant activity (Yaqub *et al*., 2016). Results are also in accordance with the results of Prakash *et al.* (2011) that the pulp extract of *Skimmia anquetilia* exhibited higher DPPH antioxidant activity than the seed extract. It is also evident that in all the four sample extracts of persimmon fruit, scavenging activity increased with increase in temperature. This is due to enhancement of the diffusion rate and the solubility of analytes in solvents. Similar trend was found by Chen *et al.* (2013) that DPPH free-radical scavenging potential increases with increase in extraction temperature in *Ficus virens* leaves.

In microwave extraction, DPPH radical scavenging activity follows the same trend as in conventional extraction that is fresh pulp extract of persimmon fruit showed significantly higher mean DPPH radical scavenging activity (77.00%), followed by fresh seed sample extract (52.93%), dried pulp sample extract (46.46%) and dried seed sample extract which showed the least scavenging activity of 30.79%. Fresh persimmon pulp and seed extracts showed higher DPPH radical scavenging activity than their dried extracts as in conventional extraction method. Similar results were found by Queiroz *et al.* (2015). The data also reveals that the mean DPPH radical scavenging activity of pulp extracts were more as compared to seed extracts. This is due to the reason that the external layers of a fruit such as peel, shell, and hull that shield the inside materials generally contain enormous quantities of functional compounds (Yaqub *et al*., 2016). With increase in microwave exposure time from 30s to 90s, DPPH free-radical scavenging activity increased significantly ($p \leq 0.05$), this may be due to the reason that heating fruits in a microwave oven increased temperature faster than other heating methods, destroying cell membranes and allowed antioxidants to be easily extracted from the fruits (Sun *et al.,* 2007). Similar results were reported by Hayat *et al.* (2010) in citrus pomace that increase in microwave treatment time significantly increased the antioxidant activity.

From Tables 1 and 2, it can be conferred that microwave-assisted extraction showed significantly ($p \leq 0.05$) higher DPPH free-radical scavenging activity than conventional extraction. This may be due to more cleavage and liberation of phenolic compounds by microwave treatment, hence resulting in the increase of free phenolic compounds and enhancement of antioxidant capacity of the extracts (Hayat *et al.*, 2010). Similar results were found by Lin *et al.* (2012) that the microwave-assisted extraction improves the extraction ability of the DPPH radical scavenging effect while working on "effects of microwave-assisted extraction on the free radical scavenging and ferrous chelating abilities of *Porphyra dentate* extract''.

In conventional extraction (Table 3), the mean ABTS antioxidant activity of fresh pulp extract were significantly higher (99.19%) than that of fresh seed sample extract (97.53%) and dried pulp extract (95.14%), while dried seed sample extract showed least average antioxidant activity of 90.72 %. This means that the ABTS scavenging activity of pulp extracts (both fresh and dried) were significantly higher than that of seed extracts. This may be because of the presence of tannins (main agent responsible for antioxidant properties) in the persimmon pulp. Similar results were found by Chodak and Tarko (2007). Also, the ABTS scavenging activity of fresh pulp (99.19%) and fresh seed (97.53%) extracts were significantly higher than their dried pulp (95.14%) and seed (90.72%) extracts. This means that the antioxidant activity of fresh extracts is greater than the dried extracts. Similar results were found by Gorinstein *et al.* (2006) that the total antioxidant activity of fresh persimmons as determined by DPPH and ABTS assays was significantly higher than that of the dried persimmons.

2,2-azinobis (3-Ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity varied significantly ($p \leq 0.05$) with extraction temperature. Highest scavenging activity was observed at 50°C extraction temperature in all the four sample extracts. Overall, an increase in ABTS activity was noticed with increase in extraction temperature from 40-50°C, however, when temperature rose from 50 to 60°C, a modest drop in ABTS was seen. This might be because relatively high temperatures have negative impacts on the bioavailability of bioactive substances. Increasing the temperature of the extraction process would facilitate extraction by improving the solubility of the solute and the diffusion coefficient, but it might also result in the thermal degradation of phenolic compounds, reducing the antioxidant properties of the crude extract (Vongsangnak *et al*., 2004; Spigno *et al*., 2007; Chew *et al*., 2011a; Chew *et al*., 2011b).

Fresh seed sample extract showed scavenging activity of 99.3%, while dried pulp sample extract showed 97.4% and dried seed sample extract showed 93.86% of ABTS activity at 60 sec microwave exposure time. Antioxidant activity of fresh sample extracts was greater than their dried extracts. The decrease in antioxidant activity in dried extracts may be ascribed to the alterations in the chemical structure of polyphenols during dehydration which cannot be extracted and determined by the available methods (Jéssica *et al*., 2013) It is also evident from the data given in Table 4 that with increase in microwave exposure time from 30-60 sec, ABTS scavenging activity enhanced, whereas a decline in ABTS activity was observed with further increase in exposure time (60-90 sec). Liu *et al.* (2013) reported an increase in ABTS antioxidant activity with increase in microwave exposure time up to 36 min and after that, antioxidant activity decreased due to degradation of antioxidant compounds.

The data presented in Tables 3 and 4 shows that the ABTS value of microwave-assisted extraction is significantly higher ($p \leq 0.05$) than that of conventional extraction. This is because of more liberation of phenolic compounds by microwave treatment. This shows the efficiency of microwave-assisted extraction over the conventional one. Gallo *et al.* (2010) while working on phenolic compounds from four different spices also found that the extracts obtained using microwaves were richer in antioxidant metabolites than those obtained by ultrasonic extraction. The findings of Pan *et al.* (2008) in "longan" peel also showed that the antioxidant of microwave extraction was superior to those of Soxhlet extraction.

It is depicted from the Table 5 that the seed sample extracts (both fresh and dried) showed the higher value of FRAP antioxidant activity than that of pulp sample extracts (fresh and dried). The fresh seed sample extract had the highest level of FRAP antioxidant activity (1209.21 μMTE/g), followed by the dried seed sample extract (1185.00 μMTE/g), the fresh pulp sample extract (634.68

μMTE/g), and the dried pulp sample extract (523.62 μMTE/g) at 60°C. This means that the results obtained in FRAP and CUPRAC assays are reverse to that of obtained in DPPH and ABTS assays. This may be due to the reason that extraction of phenolic compounds from plant material is influenced by various parameters such as extraction procedures and conditions, solvent polarity and particle size. Similar results was also found by Lee *et al.* (2006) that the seed and calyx extracts showed significantly ($p \leq 0.05$) higher antioxidant activities and phenolic contents than peel and flesh extracts while working on the "antioxidant and antigenotoxic activities of different parts of persimmon (*Diospyros kaki* cv. Fuyu) fruit". Assefa and Keum (2016), reported that the FRAP (mgTE/ 100 g DW) antioxidant activity of fresh seed extract (207.2 ± 1.9) is greater than the fresh pulp extract (132.6 ± 7.2). Similar results were also reported by Goulart *et al.* (2012) that the FRAP (mmol Trolox.g^{-1}) antioxidant activity of seed extracts of two exotic Brazilian fruits (Siriguela and Umbu) is greater than their pulp extracts. Moreover, it was observed from the table that the FRAP antioxidant activity of fresh extracts both pulp and seed are greater than the dried extracts. Antioxidant activity of fresh pulp (628.81 μMTE/g) is greater than the dried pulp extract (513.47 μMTE/g) and the antioxidant activity of fresh seed extract (1121.38 μMTE/ g) is greater than the dried seed extract (1061.44 μMTE/g). The reason for this may be some of the compounds which have significant role in the inhibition of radicals may have been destroyed in dried samples. Similar results were obtained by Capanoglu *et al.* (2013) that the FRAP antioxidant activity of fresh mulberry is significantly greater than the dried mulberry.

The FRAP antioxidant activity increased in all the four sample extracts of persimmon fruit with increase in extraction temperature. This might be because rising temperatures might encourage phenolic extraction by enhancing the compounds' solubility in extraction solvent and diffusion coefficient (Al-Farsi and Lee, 2008). It is evident from the data presented in Table 5 that the FRAP antioxidant activity of fresh pulp sample extract varied from 623.6 to 634.68 μMTE/g, in dried pulp extract 498.66 to 523.62 μMTE/g, in fresh seed extract 1017.43 to 1209.21 μMTE/g, 949.66 to 1185.00 μMTE/ g in dried seed extract when temperature was increased from 40°C to 60°C. Similar trend in FRAP activity with increase in extraction temperature was also reported by Xu *et al.* (2012) in tea (*Camellia sinensis* L.) fruit peel. The results are in accordance with the findings of Katalinic *et al.* (2004) who reported that higher temperature results in higher antioxidant capacity as determined by FRAP.

It is evident from the Table 6 that the antioxidant activity varied with fruit part, fruit type and extraction time. In fresh pulp extract the antioxidant activity varied from 744.00 to 1253.93 μMTE/g, in dried pulp extract it varied from 703.1 to 1160.00 μMTE/g, in fresh seed it varied from 1058.3 to 1259.9 μMTE/g and in dried seed extract it varied from 1008.7 to 1191.13 μMTE/g when extraction time increased from 30-90 sec. It is depicted that the highest antioxidant activity was found in fresh seed extract (1162.66 μMTE/ g) after that dried seed extract (1106.57 μMTE/g) than fresh pulp extract (1053.04 μMTE/g) and the dried pulp extract showed the least (1006.83 μMTE/g) antioxidant activity. Because polyphenol oxidase or the thermal destruction of phenolic compounds have the potential to produce oxidative breakdown, fresh sample extracts of both the pulp and the seed have higher antioxidant activity than their dried sample extracts (Shahidi and Naczk, 2004). Also from the data presented in Table 6, it is clear that with increase in extraction time, the antioxidant activity showed an increasing trend. Khizr *et al.* (2010) reported that the reducing power of kinnow pomace was increased when heated for 15 min at 250 W because of the liberation and activation of bound phenolics and therefore enhance the antioxidant activity. Similar kind of trend has been earlier reported by Xu *et al.* (2012).

Comparing the data given in Tables 5 and 6, it is clear that the FRAP antioxidant activity of microwave-assisted extracts was significantly higher than the FRAP antioxidant activity of conventional extracts of different samples of persimmon fruit. Maximum antioxidant activity was shown by fresh seed sample extract under microwave extraction for an exposure time of 1.5 min. While researching microwave-assisted simultaneous extraction of luteolin and apigenin from tree peony pod and assessment of its antioxidant activity, Wang *et al.* (2014) discovered that the FRAP antioxidant activity of extract obtained by microwave-assisted extraction was higher than those obtained by heat reflux extraction and maceration extraction.

It is evident from the Table 7 that the antioxidant activity of fresh seed sample extract is found to have highest mean antioxidant activity of 1008.29 μ moles TE/g at 60°C, followed by dried seed sample extract (890.24 μ moles TE/g) and fresh pulp sample extract (479.43 μ moles TE/g). Dried pulp sample extract showed the least average antioxidant activity of 457.47 μ moles TE/g. The antioxidant activity of seed sample extracts both fresh and dried are greater than the pulp extracts. Similar results are also depicted from the research work of Goulart *et al.* (2012) that the CUPRAC (mmol Trolox.g^{-1}) antioxidant activity of seed extracts of two exotic Brazilian fruits (Siriguela and Umbu) is greater than their pulp extracts because non-edible parts of exotic fruits have shown important antioxidant activities. It is also depicted from the Table 7 that the CUPRAC antioxidant activity of fresh extracts both pulp and seed are greater than the dried extracts. Antioxidant activity of fresh pulp (μMTE/g) is greater than the dried pulp extract (μMTE/ g) and the antioxidant activity of fresh seed extract (μMTE/g) is greater than the dried seed extract (μMTE/g). It is also evident from the data that with increase in extraction temperature from 40°C to 60°C, there was significant increase in CUPRAC antioxidant activity. This could be due to the liberation of more phenolic compounds by high heat treatment. Same results were also shown by Sahin *et al.* (2013) in *Artemisia absinthium* while increasing extraction temperature.

The mean antioxidant activity was found significantly higher in case of fresh seed sample extract (1035.99 μmoles TE/g), followed by dried seed sample extract (963.56 μmoles TE/g), fresh pulp sample extract (841.38 μmoles TE/g) with dried pulp sample extract having the lowest value of 795.35 μmoles TE/g. Also CUPRAC antioxidant activity of fresh extracts both pulp and seed are greater than their dried extracts. This indicates that the persimmons have different antioxidant activities depending on parts, solvent and cultivars. From the data presented in Table 8, it can also be seen that with increase in extraction time from 30-90 sec, the CUPRAC antioxidant activity of different samples of persimmon fruit extracts showed an increasing trend similar to previous antioxidant assays (DPPH, FRAP).

This may be due to more release of phenolic compounds into more extractable form for long extraction time at high microwave energy. However, the irradiation time cannot be increased above certain limits as longer duration of extraction time and high power of microwave degrades the phenolic compounds from plants (Song *et al.,* 2011; Narkprason *et al.,* 2015).

Comparing the values of CUPRAC for both conventional and microwave-assisted extraction methods, it is evident that microwave-assisted extraction presents more efficient method for antioxidant extraction of different samples of persimmon fruit extracts. This may be because of the molecular vibration of radiated water molecules and also dissipation factor of material and solvent produces frictional heat that affects internal temperature, increasing polyphenolic and antioxidant capacity. Setiawan *et al.* (2013) also reported microwave extraction as more advantageous method for antioxidant recovery than Soxhletation.

5. Conclusion

From the study, we may conclude that the antioxidant properties of wild persimmon are due to its polyphenolic compounds and hence can act as a natural source of antioxidants. Best recovery of antioxidants was obtained when extraction was carried out for 90 sec and 60°C in microwave and conventional extraction system, respectively. The FRAP technique depicted high reproducibility and was more rapid and simple, therefore it would be appropriate technique for determining antioxidants in persimmon fruit and seed extract.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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