# Phenolic Content, Flavonoid Content and Antioxidant Efficacy of *Opuntia elatior* Mill.

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#### ABSTRACT

Background: The current study aims at examining the unripened and ripened fruits of Opuntia elatior Mill. for its phytochemical constituents and antioxidant potential. Materials and Methods: The extracts of unripened and ripened fruits of O. elatior were prepared using various solvent systems (aqueous, ethanol, methanol, and acetone) and the extracts were evaluated for phenolics and flavonoid content as well as antioxidant activity. The antioxidant activity of the extracts was assessed by using Ferric reducing antioxidant power assay, ferrous ion chelating activity, phosphomolybdenum reducing power assay and hydroxyl radical scavenging assay. Results: The results of the study revealed that among the unripened and ripened fruits of O. elatior analyzed, unripened fruits yielded highest content of phenolics and flavonoids as compared to ripened fruits at fresh weight as well as dry weight basis. The extracts were also found to have significantly different levels of antioxidant activities in different antioxidant methods. The antioxidant activity varied in ripened and unripened fruits as well as among the solvents in different assays. The overall results indicated that the dried fruits of O. elatior having highest amount of phenolics and flavonoids also exhibited highest antioxidant activity as compared to fresh fruits. Significantly, a positive correlation was observed between the phytochemical compounds and antioxidant activity. Conclusion: The study specified that dried fruits of O. elatior showed highest phenolics and flavonoid content as well as highest antioxidant activity. Thus, dried fruits of O. elatior can be used as an accessible source of natural antioxidants with consequent health benefits.

Keywords: Opuntia, Cactaceae, Phenolics, Flavonoids, Free Radicals, Antioxidants.

# **INTRODUCTION**

Opuntia (prickly pear) is a genus of xerophytes belonging to the family Cactaceae, growing luxuriantly in the arid parts of the world. Recently, the pears of Opuntia have been discovered to contain biologically active compounds. Owing to their high nutritional value, in terms of dietary fiber, polyunsaturated fatty acid-rich oil, minerals, proteins and an assortment of other phytochemicals, the pears are gaining popularity as exotic, gourmet diet and have emerged multiple health benefits viz. antioxidant, anti-inflammatory, anti-diuretic, hypocholesterolemic, anti-diabetic, anti-proliferation, immunostimulatory and anti-ulcerogenic activity.<sup>1</sup> For centuries, these pears have been the staple diet of the native Americans. Also, these pears have been appreciated by them for their pharmacological properties. Many indigenous communities have used the pears as ethnomedicine for various ailments viz. asthma,



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inflammation, ulcer and diabetes. Prickly pear is traditionally used by Pima Indians, residing in Central and Southern Arizona, as a dietary nutrient against diabetes.<sup>2</sup> Opuntia elatior, is an important species of the genus Opuntia, used as medicine for various ailments due to beneficial health promoting properties. Almost all parts of the plant are used in traditional medicinal system for curing various diseases. The plant is digestive, carminative, diuretic and purgative; good for bronchitis of children, leucoderma, enlarged spleen, urinary burning, vesicular calculi and ophthalmia. Fruits are recommended as an expectorant and remedy for whooping cough, asthma and gonorrhea.<sup>3</sup> The fruits possess anti-asthmatic property due to its bronchodilator, mast cell stabilization, anti-inflammatory and reduction of neutrophil adhesion property. Furthermore, peel extracts of fruit have antimicrobial property against bacteria and fungi.<sup>4</sup> Despite many reports of medicinal values of the species O. elatior, little information is available for its antioxidant potential. Thus, the present study aims at quantitative analysis of phytochemicals and antioxidant potential of O. elatior. In addition to this O. elatior grow automatically in the barren region and it does not require any maintenance to survive. Raw material of this plant is easily available; in short it is highly economical. Even though easy, wide

and cheap availability of *O. elatior*, it is not extensively used in the medicine. In this contest the current study aims at investigating the therapeutic worth of *O. elatior*. So that local community and common man can explore the benefits of this plant.

# **MATERIALS AND METHODS**

## **Plant material**

The fruits of *Opuntia elatior* were used as basic materials in this study, were collected from its wild habitat from Dharward, Karnataka. The herbaria for voucher specimen of the *O. elatior* was prepared and deposited in the Department of Botany, Karnataka State Akkamahadevi Women's University, Vijayapura (Karnataka), India.

#### **Reagents and standards**

Folin-Ciocalteu reagent, aluminum chloride, ferric chloride, hydrogen peroxide, Potassium ferricyanide, sodium phosphate (monobasic and dibasic), sodium carbonate, 2,4,6-tripyridyl-s-triazine (TPTZ), ferrozine, ferrous chloride, ammonium molybdate, sodium phosphate and sulphuric acid, 1,10- phenanthroline, ascorbic acid, tannic acid, rutin, acetone, ethanol and methanol (HPLC grade) were procured from HiMedia Chemical Co. Mumbai, (India). All the solvents used during the study were of AR grade.

## **Preparation of extract**

Variation in extraction methods is usually depend on the length of the extraction period, solvent used, pH of the solvent, temperature, particle size of the plant tissues and the solvent-to-sample ratio. The basic principle is to grind the plant material (fresh or dry) finer, which increases the surface area for extraction thereby increasing the rate of extraction. In this study the extraction of plant material was achieved by homogenizing the fresh and dry plant tissue in various solvents. Earlier studies reported that solvent- to-sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal.5 The extraction method that has been widely used by researchers is plant tissue homogenization in solvent.6 Dried or wet, fresh plant parts are ground in a blender to fine particles, put in a 5 mL of solvent and shaken vigorously for 5-10 min and left for 24 hr in a shaking machine after which the extract is filtered. Then the extracts obtained were centrifuged at 8,000 xg for 15 min. The supernatant was collected and the residue was again suspended by adding 5 mL of solvents and centrifuged to complete the extraction. The supernatants pooled and the volume was adjusted to 10 mL by dilution of more distilled water. Same procedure was followed for the preparation of other solvent extracts (methanol, ethanol and acetone). All the extracts were kept at 4°C and for the assays 1% (v/v) extracts (diluted with double distilled water or respective solvents) were used.

#### Total phenolic content

Total Phenolic Content (TPC) of the plant extracts were determined using Folin-Ciocalteu method.<sup>7</sup> The reaction mixture was prepared by mixing an aliquot of the extracts (0.125 mL) with Folin-Ciocalteu reagent (0.125 mL) and 1.25 mL of saturated Na<sub>2</sub>CO<sub>3</sub> solution. The reaction mixture was thereafter incubated for 90 min at room temperature and the absorbance was measured at 760 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. A calibration curve was prepared, using a standard solution of tannic acid (10 to 100  $\mu$ g/mL, r<sup>2</sup>=0.973) and the results were expressed as mg Tannic Acid Equivalents (TAE)/ g of sample.

#### **Total flavonoid content**

Total Flavonoid Content (TFC) of the plant extracts were analyzed according to the spectrophotometric method.<sup>8</sup> The reaction mixture was prepared by adding 1.5 mL of extract to 1.5 mL of 2% methanolic AlCl<sub>3</sub>, incubated for 10 min at room temperature and the absorbance was measured at 420 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was used for the standard solution of rutin and a calibration curve was prepared (10 to 100 µg/mL,  $r^2$ =0.986). The results were expressed on fresh weight (fw) and dry weight (dw) basis as mg Rutin Equivalents (RE) / g of sample.

## Ferric reducing antioxidant power assay

The Ferric Reducing Antioxidant Power (FRAP) ability of the plant extracts was measured using a modified version of the method described by Grochowski *et al.*<sup>9</sup> An aliquot (100  $\mu$ L) of extract was added to 3 mL of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM FeCl<sub>3</sub>. 6H<sub>2</sub>O solution) and the reaction mixture was incubated at 37°C for 15 min. After that, the absorbance was measured at 595 nm. A calibration curve was prepared, using an aqueous solution of ascorbic acid (100 to 1000  $\mu$ M, r<sup>2</sup>=0.886) and the results were expressed as micromoles of ascorbic acid equivalent per gram of sample.

## Ferrous ion chelating activity

The chelating activity of the extracts for ferrous ions was measured according to the method devised by Taroreh *et al.*<sup>10</sup> To 0.5 mL of extract, 1.6 mL of deionized water and 0.05 mL of FeCl<sub>2</sub> (2 mM) was added. After 30 s, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min incubation at room temperature, the absorbance of the Fe<sup>2+</sup>-ferrozine complex was measured at 562 nm. The percentage of chelating activity of the extract was determined by using the following equation.

% chelating activity=A0-A1/A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the sample.

#### Phosphomolybdenum reducing power assay

The antioxidant activity of the extracts was assessed by the phosphomolybdenum reduction assay according to Siddeeg *et al.*<sup>11</sup> An aliquot of 0.3mL of sample solution was mixed with 3 mL of the reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped with aluminium foil and incubated at 95°C for 90 min, after incubation the tubes were cooled to room temperature and the absorbance of aqueous solution was measured at 695 nm. For reference, ascorbic acid was used and a calibration curve was prepared (10 to 100 µg/mL,  $r^2$ =0.984). The reducing capacity of the extracts was expressed as the Ascorbic Acid Equivalents (AAE) per gram of sample.

## Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was measured by following the method described by Keshari *et al.*<sup>12</sup> 0.1 mL of test sample was mixed with 0.3 mL phosphate buffer and 0.6 mL of 2 mM  $H_2O_2$ . The mixed solution was incubated for 10 min and the absorbance was recorded at 230 nm. The percent scavenging activity of the sample extracts was measured by using the formula of inhibition percentage as applied for ferrous ion chelating activity.

## **Statistical analysis**

Experimental results were statistically analyzed and expressed as mean±standard deviation. All measurements were replicated three times. Data were subjected to different statistical analysis using MS Excel and GraphPad InStat software.

## RESULTS

## **Phenolics and Flavonoid content**

The amount of total phenolics and total flavonoids present in the different extracts of O. elatior fruits were investigated (Table 1). The content of total phenolics is expressed as mg Tannic Acid Equivalent/g of plant material (TAE) and the content of total flavonoids as mg Rutin Equivalent/g of plant material (RE). Significantly highest results were found in the order aqueous>m ethanol>acetone>ethanol for total phenolics in unripened fruit and aqueous > acetone>methanol>ethanol for ripened fruits at fresh weight basis. At dry weight basis the trend was acetone> methanol>aqueous>ethanol in unripened fruit and acetone>me thanol>ethanol>aqueous in ripened fruit (Table 1). The aqueous extract was found to be having the maximum content of total phenolics in unripened (26.1mg TAE/g fw) and ripened (25.2 mg TAE/g fw) fruits at fresh weight basis and acetone extract had the highest content of total phenolics in unripened (67.5 mg TAE/g dw) and ripened (52.3 mg TAE/g dw) fruits at dry weight basis.

The content of flavonoids is also determined in the fruit extracts of *O. elatior* (Table 1). The flavonoid content was found highest in the order acetone>ethanol>methanol>aqueous in unripened fruit and aqueous>methanol>acetone>ethanol in ripened fruits at fresh weight basis. At dry weight basis the trend was methan ol>acetone>ethanol>aqueous in unripened fruit and acetone>et hanol>methanol>aqueous in ripened fruit. The acetone extract was found to be having the maximum content of total flavonoids in unripened fruit (0.45 mg RE/g fw) and aqueous extract had highest flavonoid content in ripened fruit (0.34 mg RE/g fw) at fresh weight basis. While at dry weight basis flavonoid content was found to be highest in methanolic extract in unripened fruit (1.77 mg RE/g dw) and in acetone extract in ripened fruit (0.74 mg RE/g dw).

#### Ferric reducing antioxidant power

The antioxidant capacities of the extracts from fruits of *O. elatior* were estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) (Table 2). The ferric reducing ability of the extracts revealed that the aqueous extracts of unripened (333.1 mM AAE/g fw) and ripened fruits (254.3 mM AAE/g fw) exhibited highest FRAP activity at fresh weight basis. While, at dry weight basis the activity was found to be highest in acetone extracts of unripened fruits (2091.1 mM AAE/g dw) and ripened fruits (1194.1 mM AAE/g dw). Amongst the dry and fresh fruit extracts, dry fruit extracts exhibited highest ferric reducing antioxidant power.

#### Ferrous ion chelating activity

The ferrous ion chelating activity of the *O. elatior* fruits was estimated at both fresh and dry weight basis. The estimated values for ion chelating activity of the extract in *O. elatior* ranged from 39.2 to 58.4% and 59.9 to 70.4% in unripened and ripened fruit extracts respectively at fresh weight basis. The values at dry weight basis varied from 49.7 to 63.6% in unripened fruit extracts (Table 2). Among the different solvents used aqueous extraction system exhibited highest chelating activity at fresh weight basis in both unripened and ripened fruits. However, at dry weight basis the chelating activity was highest in acetone and methanol extracts in unripened and ripened fruits respectively.

#### Phosphomolybdenum reducing power assay

The ranking of the antioxidant capacity obtained by this method, displayed that the antioxidant activity of the extract varied in unripened (1.71 to 2.34 mg AAE/g fw) and ripened fruits (2.30 to 3.34 mg AAE/g fw) at fresh weight basis (Table 3). Whereas at dry weight basis the values ranged from 4.94 to 6.28 mg AAE/g dw in unripened fruit extracts and 6.20 to 8.79 mg AAE/g dw in ripened fruit extracts. Among the different solvent extracts aqueous extract exhibited highest phosphomolybdenum reduction in both unripened (2.34 mg AAE/g fw) and ripened

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Solvents	Plant material	Total Phenolics (mg TAE/ g fresh/ dry weight)		Total Flavonoids (mg RE/g fresh/ dry weight)		
		Unripened fruit	Ripened fruit	Unripened fruit	Ripened fruit	
Aqueous	Fresh	26.1±0.41	25.2±0.04	0.34±0.01	0.29±0.01	
	Dry	49.2±0.48	42.9±0.04	0.62±0.01	0.47±0.06	
Methanol	Fresh	25.3±0.05	21.3±0.04	0.36±0.01	0.27±0.02	
	Dry	63.1±0.04	49.6±0.04	$1.77 \pm 0.04$	0.54±0.09	
Ethanol	Fresh	23.5±0.51	20.1±0.04	$0.42 \pm 0.02$	0.12±0.08	
	Dry	$48.9 \pm 0.08$	46.7±0.02	0.97±0.01	0.61±0.13	
Acetone	Fresh	23.6±0.81	22.1±0.48	$0.45 \pm 0.02$	0.24±0.01	
	Dry	67.5±0.08	52.3±0.48	$1.55 \pm 0.04$	$0.74 \pm 0.14$	

#### Table 1: Total phenolics and flavonoid content in fruits of Opuntia elatior Mill.

Values are expressed as mean±SD of triplicate measurements.mg TAE/ g dry weight: milligram tannic acid equivalent per gram dry weight.mg RE/g dry weight: milligram rutin equivalent per gram dry weight.

Table 2: Antioxidant potential of	f different extracts obtained	from fruits of Opuntia elatior Mill.
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Solvents	Plant material	Ferric reducing a (mM AAE /g fre	ntioxidant power esh/ dry weight)	Ferrous ion chelating Activity (%)		
		Unripened fruit	Ripened fruit	Unripened fruit	Ripened fruit	
Aqueous	Fresh	333.1±19.4	254.3±1.94	57.58	70.46	
	Dry	1387.3±1.76	997.5±5.71	58.41	87.94	
Methanol	Fresh	297.9±1.36	231.8±1.98	51.70	59.98	
	Dry	1994.5±1.49	1176.1±1.68	63.56	89.15	
Ethanol	Fresh	292.6±1.36	189.1±3.37	39.28	60.41	
	Dry	1777.3±20.4	1097.6±2.98	49.77	78.24	
Acetone	Fresh	322.1±2.96	234.1±1.94	41.31	70.19	
	Dry	2091.1±3.24	1194.1±2.46	63.66	81.61	

Values are expressed as mean±SD of triplicate measurements.mM AAE /g dry weight: milli molar ascorbic acid equivalent per gram dry weight.

#### Table 3: Antioxidant potential of different extracts obtained from fruits of Opuntia elatior Mill.

Solvents	Plant material	Phosphomolybden (mg AAE /g fre	um reducing power sh /dry weight)	Hydroxyl radical scavenging activity (%)		
		Unripened fruit	Ripened fruit	Unripened fruit	Ripened fruit	
Aqueous	Fresh	2.34±0.02	3.34±0.01	88.52	78.21	
	Dry	4.94±0.19	6.20±0.03	94.74	84.62	
Methanol	Fresh	1.79±0.03	2.30±0.02	92.41	80.62	
	Dry	6.28±0.01	7.19±0.02	95.91	86.21	
Ethanol	Fresh	1.76±0.02	2.37±0.01	85.99	70.81	
	Dry	5.42±0.02	6.35±0.05	93.91	84.94	
Acetone	Fresh	1.71±0.03	2.38±0.02	91.82	82.85	
	Dry	5.87±0.02	8.79±0.04	94.94	90.85	

Values are expressed as mean±SD of triplicate measurements.mg AAE /g dry weight: milligram ascorbic acid equivalent per gram dry weight.

fruits (3.34 mg AAE/g fw) at fresh weight basis. At dry weight basis methanol extract in unripened fruit (6.28 mg AAE/g dw) and acetone extract in ripened fruit (8.79 mg AAE/g dw) showed highest reducing power.

#### Hydroxyl radical scavenging activity

The hydroxyl radical scavenging potential of various solvent extracts of *O. elatior* fruits is shown in Table 3. Interpretation of the table revealed that each extract of unripened and ripened

Antioxidant	Unripened fruit		<b>Ripened fruit</b>		Unripened fruit		Ripened fruit	
activity	Fresh weight		Fresh weight		Dry weight		Dry weight	
	ТРС	TFC	ТРС	TFC	ТРС	TFC	ТРС	TFC
FRAP	0.998**	0.875	0.763	0.378	0.806	0.518	0.922*	0.416
Fe <sup>2+</sup> chelation	0.993**	0.871	0.617	0.940*	0.699	0.484	0.201	0.759
MoO <sub>2</sub> P reduction	0.996**	0.940*	0.853*	0.570	0.470	0.797	0.968*	0.748
OH. Scavenging	0.614	0.336	0.841	0.582	0.475	0.805	0.960*	0.842

Table 4: Comparison between phytochemical constituents and different antioxidant assays as represented by correlation coefficient.

Data were statistically analyzed using Pearson correlation coefficient test. \*Indicates a significant difference at the level of p < 0.05.\*\*Indicates a significant difference at the level of p < 0.01.

fruit display hydroxyl radical scavenging activity and the activity observed was in the range of 85.9 to 92.4% and 70.8 to 82.8% in unripened fruits and ripened fruits respectively at fresh weight basis. The hydroxyl radical scavenging activity at dry weight basis ranged from 93.9 to 95.9% in unripened fruit and from 84.6 to 90.8% in ripened fruit. Here, methanol extract in unripened fruit (95.9%) and acetone extract in ripened fruit (90.8%) exhibited highest radical scavenging activity.

## Comparison between phytochemical constituents and different antioxidant assays by correlation analysis

To find the relationship between the antioxidant activity, total phenolics and flavonoid content, we performed linear regression and correlation analyses of the values of total antioxidant capacity by FRAP, Fe<sub>2</sub>+ chelating activity, MoO<sub>2</sub>P reduction and OH. scavenging activity and also with the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). The correlations of TPC and TFC against the antioxidant activity based on the FRAP, Fe<sup>2+</sup> chelation, MoO<sub>2</sub>P reduction and OH scavenging assays in the fruits of O. elatior were significant (Table 4). In fresh unripened fruits a strong correlation was found between TPC against FRAP (0.998), Fe<sup>2+</sup> chelation (0.993) and phosphomolybdenum reduction (0.996), but no such significant correlation was found between TPC against OH scavenging activity. While in fresh ripened fruits TPC correlated significantly with phosphomolybdeum reducing power (0.853) but was not significantly correlated with FRAP, Fe<sup>2+</sup> chelation and OH scavenging activity. In case of unripened dry fruits TPC exhibited a good correlation with FRAP (0.806), while with Fe<sup>2+</sup> chelation, phosphomolybdeum reducing power and OH scavenging activity a fair correlation was revealed. In ripened dry fruits TPC showed a strong correlation with FRAP (0.922), phosphomolybdeum reducing power (0.968) and OH scavenging activity (0.960) but was not significantly correlated with  $Fe_2$ + chelation activity (0.201).

In addition, the content of Total Flavonoids (TFC) showed a good correlation with most of the antioxidant assays. In case of fresh unripened fruits TFC with phosphomolybdeum reducing power (0.940) showed a good correlation but was not significantly correlated with FRAP,  $Fe_2$ + chelation activity and OH scavenging activity. For fresh ripened fruits the correlation of TFC with FRAP were not significant but TFC exhibited a significant correlation with  $Fe^{2+}$  chelation activity (0.940), but the correlations of TFC against the phosphomolybdeum reducing power and OH scavenging activity were satisfactory. In dry unripened fruits, a good correlation was found between TFC against OH scavenging activity with coefficient of correlation 0.805. In case of dry ripened fruits TFC correlated significantly with OH. scavenging activity (0.842) but the correlations of TFC with FRAP, phosphomolybdeum reduction and OH scavenging activity was not found significant.

## DISCUSSION

Phenolic substances and flavonoids have been shown to be responsible for the antioxidant activity of many of the plants.<sup>13</sup> The results of the TPC and TFC analyses showed that the extracts from the fruits of *O. elatior* had maximum phenolics and flavonoids at dry weight basis than that of fresh weight basis. Overall, from these observations it was revealed that the dry plant extracts yield maximum amount of phenolics and flavonoids than fresh extracts. Fresh plant extracts may contain lower amounts of bioactive principles due to a water content of typically 75 to 95%, resulting in a marked dilution effect.<sup>14</sup>

The FRAP assay measures the ability of antioxidants to reduce the ferric 2, 4, 6-tripyridyl-S-triazine complex [Fe(III)-(TPTZ)2]2+ to intensely blue colored ferrous complex [Fe(II)-(TPTZ)2]2+ in acidic medium.<sup>15</sup> FRAP values of the *O. elatior* fruit extracts are calculated by measuring the absorbance at 595 nm and relating it to the standard ascorbic acid. The results displayed the reduction capacity of the fruit extracts both at dry weight and fresh weight basis, but the reduction ability was highest in dry fruit extracts as compared to fresh. Thus, the observed reduction ability of the *O. elatior* fruit extracts may serve as a significant indicator of its potential antioxidant activity.

The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease.<sup>16</sup> Because  $Fe^{2+}$  causes the production of oxyradicals and lipid peroxidation, minimizing its concentration affords protection against oxidative damage. In the presence of other chelating agents, the ferrozine complex formation is disrupted with the result that the red color of the complexes decreases, therefore allows estimation of the chelating activity of the coexisting chelator.<sup>17</sup> The ability to chelate ferrous ions was higher in ripened fruits of *O. elatior*, which indicates that ripened fruit extracts chelate the iron ions more than that of unripened fruit extracts.

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/ Mo (V) complex with the maximal absorption at 695 nm.<sup>18</sup> Being simple and independent of other antioxidant measurements commonly employed, the assay was extended to plant polyphenols. Amongst the fruits, ripened fruits seem to be having a higher capacity than unripened fruits and the antioxidant activity was highest in dry fruit extracts as compared to that of fresh fruit extracts. This may be explained by the fact that the transfer of electrons/ hydrogen from antioxidants depends on the structure of the antioxidants.<sup>19</sup>

Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides.<sup>20</sup> Hydroxyl radical is involved in lipid peroxidation which affects membrane fluidity, enzymes and receptors activity leading to apoptosis. If hydroxyl radical is generated near nucleic acids it reacts with purine and pyrimidine bases and 2-deoxyribose, leading to mutations which play an important role in carcinogenesis, as well as in neurodegenerative and cardiovascular diseases.<sup>21</sup> Thus, removing OH is very important for the protection of living systems. The hydroxyl radical scavenging activity of the extracts has been found highest in unripened fruits as compared to ripened fruits, while in comparison with fresh and dry fruit extracts, the extracts prepared at dry weight basis exhibited highest hydroxyl radical scavenging activity.

Since, the extracts from various samples have the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction; they could serve as potential antioxidants. The presence of phenolics including flavonoids have been reported in the present study and correlated with the antioxidant activities reported. The correlation patterns indicated an unpredictable correlation; the positive correlation indicated that the phenolics and flavonoids contribute to the antioxidant activity of *O. elatior.* While, the negative correlation between TPC, TFC and antioxidant activity suggested that it could be related to other antioxidant compounds contained in the fruit. However, the significant positive correlations observed showed

that the extract of *O. elatior* which contains highest amount of phenolics and flavonoid compounds exhibited the greatest antioxidant activity.

## CONCLUSION

The results of the current study revealed that the *O. elatior* fruit has high phenolics and flavonoid content as well as strong antioxidant potential. The results indicated that the dried fruits of *O. elatior* contains substantial amount of phenolics and flavonoids as compared to the fresh fruits and it is the extent of phenolics present in this extract being responsible for its marked antioxidant activity as assayed through various antioxidant models. Thus, it can be concluded that dried fruit extracts of *O. elatior* can be used as an accessible source of natural antioxidants with consequent health benefits. This offers opportunities to formulate value added products from fruits, nutraceuticals and food applications to enhance health benefits. Thus, the research reported herein raised new questions and new opportunities for further research.

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## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

#### ABBREVIATIONS

**TPTZ:** 2, 4, 6-tripyridyl-s-triazine; **TPC:** Total phenolic content; **TAE:** Tannic acid equivalents; **TFC:** Total flavonoid content; **fw:** fresh weight; **DW:** Dry weight; **RE:** Rutin equivalents; **FRAP:** Ferric reducing antioxidant power; **AAE:** Ascorbic acid equivalents.

#### REFERENCES

- 1. Patel S. Reviewing the prospects of *Opuntia* pears as low-cost functional foods. Rev Environ Sci Bio Technol. 2013;12(3):223-34. doi: 10.1007/s11157-012-9295-6.
- Wolfram R, Budinsky A, Efthimiou Y, Stomatopoulos J, Oguogho A, Sinzinger H. Daily prickly pear consumption improves platelet function. Prostaglandins Leukot Essent Fatty Acids. 2003;69(1):61-6. doi: 10.1016/s0952-3278(03)00057-7, PMID 12878452.
- 3. Yusuf M, Begum J, Hoque MN, Chowdhary JU. Medicinal plants of Bangladesh. Bangladesh: Bangladesh Council of Scientific and Industrial Research; 2009.
- Chauhan SP, Sheth NR, Jivani NP, Rathod IS, Shah PI. Biological actions of Opuntia species. Syst Rev Pharm. 2010;1(2):146-51. doi: 10.4103/0975-8453.75064.
- Green RJ. Antioxidant activity of peanut plant tissues [masters thesis]. North Carolina State University; 2004.
- Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk J Biol. 2005;29:203-10.
- Lee YH, Choo C, Watawana MI, Jayawardena N, Waisundara VY. An appraisal of eighteen commonly consumed edible plants as functional food based on their antioxidant and starch hydrolase inhibitory activities. J Sci Food Agric. 2015;95(14):2956-64. doi: 10.1002/jsfa.7039, PMID 25491037.
- Ramos RTM, Bezerra ICF, Ferreira MRA, Soares LAL. Spectrophotometric quantification of flavonoids in herbal material, crude extract, and fractions from leaves of *Eugenia uniflora* Linn. Pharmacogn Res. 2017;9(3):253-60. doi: 10.4103/pr.pr\_143\_16, PMID 28827966.
- 9. Grochowski DM, Uysal S, Aktumsek A, Granica S, Zengin G, Ceylan R, et al. *In vitro* enzyme inhibitory properties, antioxidant activities, and phytochemical profile of

*Potentilla thuringiaca*. Phytochem Lett. 2017;20:365-72. doi: 10.1016/j.phytol.2017. 03.005.

- Taroreh M, Raharjo S, Hastuti P, Murdiati A. Antioxidative activities of various fractions of Gedi's leaf extracts (*Abelmoschus Manihot* L. Medik). Agric Agric Sci Procedia. 2016;9:271-8. doi: 10.1016/j.aaspro.2016.02.112.
- Siddeeg A, AlKehayez NM, Abu-Hiamed HA, Al-Sanea EA, Al-Farga AM. Mode of action and determination of antioxidant activity in the dietary sources: an overview. Saudi J Biol Sci. 2021;28(3):1633-44. doi: 10.1016/j.sjbs.2020.11.064, PMID 33732049.
- 12. Keshari AK, Srivastava A, Verma AK, Srivastava R. Free radicals scavenging and protein protective property of *Ocimum sanctum* (L). J Pharm Res Int. 2017;14(4):1-10. doi: 10 .9734/BJPR/2016/31445.
- Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel). 2018 Aug 25;5(3):93. doi: 10.3390/medicin es5030093, PMID 30149600.
- Bone KA. Clinical guide to blending liquid herbs. Edinburgh: Churchill Livingstone; 2003.
- Nwachukwu ID, Aluko RE. Structural and functional properties of food protein-derived antioxidant peptides. J Food Biochem. 2019;43(1):e12761. doi: 10.1111/jfbc.12761, PMID 31353492.

- 16. Engwa GA. Free Radicals and the Role of Plant Phytochemicals as Antioxidants against oxidative stress-related diseases. InTech. 2018. doi: 10.5772/intechopen.76 719.
- Wannes WA, Marzouk B. Characterization of myrtle seed (*Myrtus communis* var. baetica) as a source of lipids, phenolics, and antioxidant activities. J Food Drug Anal. 2016;24(2):316-23. doi: 10.1016/j.jfda.2015.11.001, PMID 28911585.
- Wan C, Yu Y, Zhou S, Liu W, Tian S, Cao S. Antioxidant activity and free radical-scavenging capacity of *Gynura divaricata* leaf extracts at different temperatures. Pharmacogn Mag. 2011;7(25):40-5. doi: 10.4103/0973-1296.75900, PMID 21472078.
- Loo AY, Jain K, Darah I. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chem. 2008;107(3):1151-60. doi: 10. 1016/j.foodchem.2007.09.044.
- Wang H, Gao XD, Zhou GC, Cai L, Yao WB. *In vitro* and*in vivo* antioxidant activity of aqueous extract from*Choerospondias axillaris* fruit. Food Chem. 2008;106(3):888-95. doi: 10.1016/j.foodchem.2007.05.068.
- 21. Rathee JS, Hassarajani SA, Chattopadhyay S. Antioxidant activity of *Mammea longifolia* bud extracts. Food Chem. 2006;99(3):436-43. doi: 10.1016/j.foodchem.20 05.08.020.

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