

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.708.299>

Studies on Physico-chemical and Antioxidant Properties of Wild Pomegranate Fruits in Different Locations of Himachal Pradesh, India

Abhimanyu Thakur*, N.S. Thakur, Hamid and Pradeep Kumar

Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan – 173230, (Himachal Pradesh), India

*Corresponding author

ABSTRACT

Keywords

Wild pomegranate, Locations, Physico-chemical characteristics, Antioxidant activity

Article Info

Accepted:

15 July 2018

Available Online:

10 August 2018

Studies were carried out on physico-chemical and antioxidant properties of wild pomegranate fruits found in hilly slopes of Himachal Pradesh. Among physical parameters, fruit size and weight were found to be highest in Basantpur location. The visual colour of mature fruits from all the locations was found to be yellowish green, whereas, the colour of arils was observed as red in all the locations with slight variations in the intensity. TSS, sugars and anthocyanins content was found higher in Karsog location, whereas acid content and ascorbic acid content were found higher in Darlaghat and Basantpur locations, respectively. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant activity, FRAP (Ferric reducing anti-oxidant power) and reducing power were recorded highest in Karsog location whereas metal chelating activity was slightly higher in Basantpur location than Karsog location.

Introduction

Pomegranate (*Punica granatum* L.), belongs to Punicaceae family and it is a popular fruit of tropical and subtropical regions. The cultivated forms are found in Iran, India and the Mediterranean countries such as Egypt, Turkey, Spain, Tunisia and Morocco, whereas, its wild form is widely distributed in Transcaucasia and Central Asia (Ercisli *et al.*, 2011 and Chandra *et al.*, 2014). In India, it is found in vast tract of the hill slopes of Himachal Pradesh, Jammu and Kashmir and Uttarakhand at an altitude of 900 to 1800 m above mean sea level. In Himachal Pradesh, it is distributed in some pockets of Solan, Sirmour, Mandi, Shimla, Kullu and Chamba

districts (Thakur *et al.*, 2011). The traditional importance of pomegranate as a medicinal plant is well established by various researchers. The edible part of the pomegranate fruit is arils, which are rich in organic acids, vitamins, sugars and bioactive compounds (Tehranifar *et al.*, 2010). The fruit is laxative, diuretic and used for curing vomiting, sore throat, brain diseases, spleen complaints, chest troubles, scabies, bronchitis, liver and kidney disorders (Kirtikar and Basu, 1935). The pomegranate juice has been found to contain polyphenols (primarily ellagic and punicalagin) that may lower down the risk of heart diseases (Aviram *et al.*, 2004) and slow down the cancer progress (Adams *et al.*, 2006). The various flavonoids like

anthocyanins, ellagic acid derivatives and hydrolysable tannins (punicalagin, gallic and ellagic acid) are responsible for the antioxidant activity of pomegranate fruit and a very high antioxidant activity in the pomegranate fruit extracts (peel, juice and seeds) have been observed by various researchers (Gill *et al.*, 2000; Aviram *et al.*, 2000 and Singh *et al.*, 2002).

As there is greater variation in physico-chemical characteristics of wild pomegranate fruits, present studies were carried out to compare various quality characteristics of wild pomegranate fruits in different locations. No work has been carried out on antioxidant properties of wild pomegranate fruits so studies were undertaken to evaluate the antioxidant potential of the fruit.

Materials and Methods

Collection of fruit and chemicals

Wild pomegranate fruits were procured from four locations of Himachal Pradesh as per the details given in Table 1. The fruits were further used for various physico-chemical analysis and the chemicals used during the entire study were procured from local market.

Physico-chemical analysis

Length and breadth of fruit samples were measured with the help of Vernier calliper. Digital weighing balance was used to weigh the fruit samples and arils. The colour of arils was observed visually by comparing with colour charts of Royal Horticulture Society, London. Weight of arils divided by weight of fruit gave aril percentage of fruit. Moisture, total solids, TSS, sugars, titratable acidity, ascorbic acid, anthocyanins and ash content of fruit as well as juice were determined according to Ranganna (1997). The pH of the samples was determined by using a digital pH

meter (CRISON Instrument, Ltd, Spain). Total phenol content was determined by Folin-Ciocalteu procedure given by Singleton and Rossi (1965). The total flavonoid content of fruit samples was estimated according to the method of Ilahy *et al.*, (2011).

Antioxidant properties

One ml of sample was dissolved in 10 ml of methanol and 0.1 ml of methanolic (1 ml/g sample in 10 ml methanol) extract was taken for the estimation of various antioxidant properties. Free radical scavenging activity was measured as per the method of Brand-Williams *et al.*, (1995). DPPH was used as a source of free radical and antioxidant activity was expressed as per cent. Metal chelating activity was determined according to method of Dinis *et al.*, (1994) and expressed as per chelation. Antioxidant activity as per FRAP assay was estimated according to the method of Benzie and Strain (1996) and expressed as $\mu\text{M Fe}^{2+}/100 \text{ g}$. Reducing power was determined as per the method of Oktay *et al.*, (2003) absorbance of the sample extract at 700 nm was taken as a measure of reducing power.

Statistical analysis

The data on physico-chemical characteristics fruits were analyzed by the Randomized Block Design (RBD). The parameters for various chemical characteristics of fruit conducted in these studies were replicated five times and for the physical characteristics were replicated twenty times.

Results and Discussion

Physical characteristics

Data of physical characteristics of wild pomegranate fruit presented in Table 2 indicate that length and breadth of fruit varied from 52.06 to 64.80 mm and 50.07 to 59.15

mm, respectively from different locations. The maximum fruit length and breadth were recorded in Basantpur location whereas, minimum was recorded in Narag location. Further, weight of fruit from various locations ranged from 73.94 to 118.83 g and maximum and minimum fruit weight were recorded in Basantpur and Narag location, respectively. The visual colour of mature fruit from all four locations was found to be yellowish green, whereas, the colour of arils was observed as red in all the locations with slight variations in the intensity. Significantly, highest (56.01 %) aril percentage was found in Karsog location and minimum (50.90 %) in Narag location.

The weight of 100 arils of fruit ranged from 12.72 to 15.42 among different locations. However, maximum weight of 100 arils was observed in Basantpur location and minimum in Narag location. Nearly similar results for these parameters of wild pomegranate fruit have also been reported by Parmar and Kaushal (1982), Singh and Kingsley (2008), Thakur *et al.*, (2010), Thakur *et al.*, (2011) and Bakshi *et al.*, (2014).

Chemical characteristics

The data pertaining to chemical characteristics of wild pomegranate fruit in different locations has been presented in Table 3.

Moisture and total solids

The highest (76.95 %) moisture content of fruit was found in Basantpur location and lowest (73.15 %) in Narag location which was statistically at par with Darlaghat location. Whereas, the total solids content of fruit at different locations varied from 23.05 to 26.85 per cent and the maximum was recorded in Narag location. Almost, similar results for moisture content of wild pomegranate fruits have been reported by Chauhan *et al.*, (1994), Thakur *et al.*, (2011) and Rawat *et al.*, (2012).

TSS and sugars

TSS of fruit ranged between 15.80 to 18.50 °B, however, highest (18.50 °B) TSS was recorded in Karsog location which was at par with Darlaghat location and minimum (15.80) in Narag location.

Reducing and total sugars content of fruit in various locations varied from 8.09 to 9.45 and 9.42 to 10.96 per cent, respectively. The maximum reducing and total sugars contents were observed in Karsog locations, whereas, minimum was recorded in Narag location. The results on above parameters of wild pomegranate fruits are near to the values reported by Parmar and Kaushal (1982), Singh and Kingsley (2008), Thakur *et al.*, (2011), Rawat *et al.*, (2012) and Wani *et al.*, (2014).

Titrateable acidity and pH

The titrateable acid content of wild pomegranate fruits ranged from 3.24 to 3.88 per cent among different locations. It was recorded maximum (3.88 %) in Darlaghat location which was statistically at par with Karsog location and minimum (3.24 %) in Narag location. The pH value of fruits among different locations varied from 2.79 to 3.09, which was recorded highest (3.09) in Narag location and lowest (2.79) in Darlaghat location.

The pH in Darlaghat location was found to be at par with Karsog location. Almost, similar results for titrateable acidity and pH of wild pomegranate fruits have been reported by Thakur *et al.*, (2010) and Thakur *et al.*, (2011).

Ascorbic acid and anthocyanins

Data pertaining to ascorbic acid content of fruits reveal that it ranged from 19.15 to 22.48 mg/100 g among different locations.

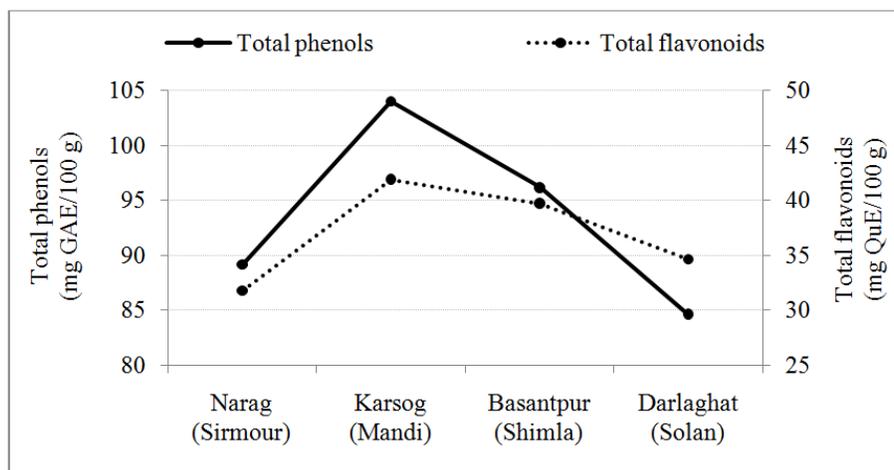


Figure.1 Total phenols (mg GAE/100 g) and flavonoids (mg QuE/100 g) of wild pomegranate fruit of different locations

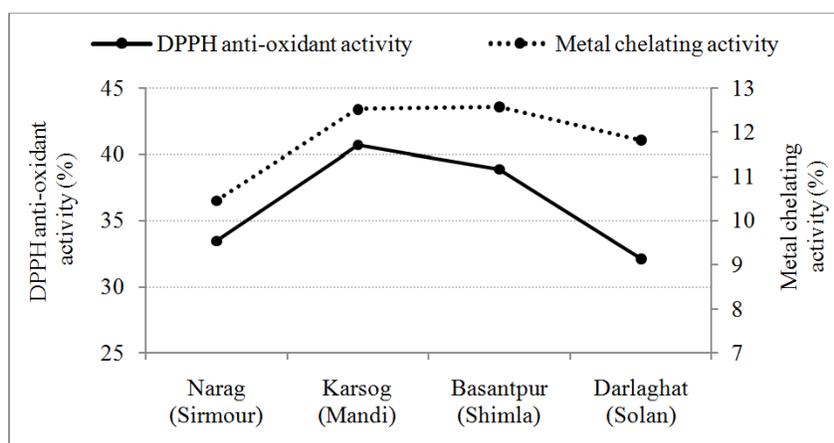


Figure.2 DPPH anti-oxidant activity (%) and metal chelating activity (%) of wild pomegranate fruit of different locations

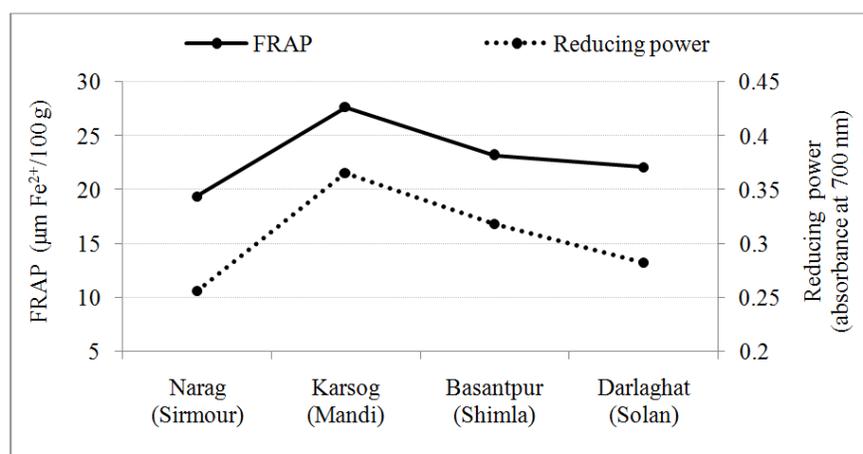


Figure.3 FRAP (µm Fe²⁺/100 g) and reducing power (abs. at 700 nm) of wild pomegranate fruit of different locations

Table.1 Details of locations for procurement of wild pomegranate fruits

Sr. No.	Location	District	Height above mean sea level (m)
1	Narag	Sirmour	1130
2	Karsog	Mandi	1265
3	Basantpur	Shimla	1325
4	Darlaghat	Solan	1390

Table.2 Physical characteristics of wild pomegranate fruit of different locations

Characteristics		Location				
		Narag (Sirmour)	Karsog (Mandi)	Basantpur (Shimla)	Darlaghat (Solan)	CD 0.05
Size	Length (mm)	52.06	56.81	64.80	60.93	2.59
	Breadth (mm)	50.07	55.24	59.15	55.67	1.98
Weight (g)		73.94	95.45	118.83	105.34	2.40
Colour of fruit		Yellowish green	Yellowish green	Yellowish green	Yellowish green	-
*Colour of arils		Red 50 B	Red 45 A	Red 45 C	Red 45 C	-
Aril (%)		50.90	56.01	52.61	54.15	1.24
Weight of 100 arils (g)		12.72	14.85	15.42	14.89	0.19

* Colour card number of Royal Horticulture Society, London

Table.3 Chemical characteristics of wild pomegranate fruit of different locations

Characteristics		Location				
		Narag (Sirmour)	Karsog (Mandi)	Basantpur (Shimla)	Darlaghat (Solan)	CD 0.05
Moisture (%)		73.15	74.89	76.95	74.31	1.22
Total solids (%)		26.85	25.11	23.05	25.69	1.22
TSS (°B)		15.80	18.50	17.20	18.00	1.28
Reducing sugars (%)		8.09	9.45	8.82	9.15	0.20
Total sugars (%)		9.42	10.96	10.05	10.73	0.17
Titratable acidity (%)		3.24	3.82	3.60	3.88	0.11
pH		3.09	2.86	2.95	2.79	0.07
Ascorbic acid (mg/100 g)		20.89	22.45	22.48	19.15	0.53
Anthocyanins (mg/100 g)		15.69	22.15	19.58	20.82	1.10
Ash (%)		0.78	0.83	0.81	0.82	0.02

The highest (22.48 mg/100 g) ascorbic acid was found in Basantpur location which was at par with Karsog location and lowest (19.15 mg/100 g) in Darlaghat location.

Our results are within the range of the results reported by Chauhan *et al.*, (1994), Singh and Kingsly (2008), Thakur *et al.*, (2011) and Wani *et al.*, (2014) in wild pomegranate fruits. The anthocyanins content of fruit

ranged from 15.69 to 22.15 mg/100 g among different locations, however, highest (22.15 mg/100 g) was found in Karsog location and lowest (15.69 mg/100 g) in Narag location. Nearly, similar results of anthocyanins content have been reported by Thakur *et al.*, (2011) and Wani *et al.*, (2014) in wild pomegranate fruits.

Ash content

The ash content of fruits ranged from 0.78 to 0.83 per cent among different locations. The highest (0.83 %) ash content was found in Karsog location which was at par with Darlaghat and Basantpur location and lowest (0.78 %) in Narag location. Nearly, similar results of ash content have been reported by Thakur *et al.*, (2011) in wild pomegranate fruits.

Antioxidant properties

Data pertaining to antioxidant properties of wild pomegranate fruits in different locations is presented in Figure 1-3.

Total phenols and flavanoids

The total phenols content of fruits in different locations varied from 84.64 to 103.97 mg GAE/100 g and maximum (103.97 mg GAE/100 g) was found in Karsog location and minimum (84.64 mg GAE/100 g) in Darlaghat location. The above results are in conformity with the findings of Thakur *et al.*, (2010) and Thakur *et al.*, (2011). The total flavonoids content of fruit varied from 31.75 to 41.89 mg QuE/100 g among different locations. It was recorded highest (41.89 mg QuE/100 g) in Karsog location and lowest (31.75 mg QuE/100 g) in Narag location. Our findings are near to the values recorded by Gadze *et al.*, (2012), Li *et al.*, (2015) and Yan *et al.*, (2017) in commercial pomegranate cultivars.

DPPH antioxidant activity and metal chelating activity

The DPPH antioxidant activity of fruit varied from 32.11 to 40.72 per cent among different locations. It was recorded highest (40.72 %) in Karsog location and lowest (32.11) in Darlaghat location followed by Narag location. The metal chelating activity of wild pomegranate fruits ranged from 10.45 to 12.57 per cent among different locations. It was recorded maximum (12.57 %) in Basantpur location which was statistically at par with Karsog location and minimum (10.45 %) in Narag location. The results on above parameters of wild pomegranate fruits are near to the values reported by Tezcan *et al.*, (2009), Tehranifar *et al.*, (2010) and Hmid *et al.*, (2013) in commercial pomegranate cultivars.

FRAP antioxidant activity and reducing power

Data pertaining to FRAP antioxidant activity assay of fruits reveal that it ranged from 19.37 to 27.65 $\mu\text{m Fe}^{2+}/100\text{ g}$ among different locations. The highest (27.65 $\mu\text{m Fe}^{2+}/100\text{ g}$) FRAP antioxidant activity was found in Karsog location and lowest (19.37 $\mu\text{m Fe}^{2+}/100\text{ g}$) in Narag location. Our findings are near to the values recorded by Akhavan *et al.*, (2015) in commercial pomegranate cultivars. The reducing power of the wild pomegranate fruit varied from 0.256 to 0.365 (absorbance at 700 nm) among different locations. It was recorded highest (0.365) in Karsog location and lowest (0.256) in Narag location. Our findings are near to the values recorded by Orak *et al.*, (2012) in commercial pomegranate cultivars.

It is thus concluded that wild pomegranate fruits have wide variation for various physical and chemical characteristics. Among physical characteristics fruit size and weight was

reported maximum at Basantpur location whereas, colour of fruit and arils was record as yellowish green and red, respectively in all the locations. Most of the chemical and antioxidant characteristics were found highest at Karsog and Basantpur locations whereas, the fruits from Narag locations were low in various quality characteristics.

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How to cite this article:

Abhimanyu Thakur, N.S. Thakur, Hamid and Pradeep Kumar. 2018. Studies on Physico-chemical and Antioxidant Properties of Wild Pomegranate Fruits in Different Locations of Himachal Pradesh, India. *Int.J.Curr.Microbiol.App.Sci.* 7(08): 2842-2850.
doi: <https://doi.org/10.20546/ijcmas.2018.708.299>