ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Elemental and Phytochemical Variation among Healthy and Cracked Fruit of *Punica granatum* L. From Selected Areas of Kachchh Region, India

Pooja Mesurani, Vijaykumar R. Ram

Abstract

Punica granatum L. also called Pomegranate is an commercially important fruit plant belonging to Punicaceae family. Cultivation for pomegranate in Kachchh district is increasing drastically but cracking of pomegranate is one of the serious problem that badly affects the production and quality of pomegranate and also economic loss to farmers. For this purpose, the study was conducted to identify elemental and phytochemicals variation in leaves among healthy and cracked fruit. Identification of elements in leaves was carried using scanning electron microscope (SEM) energy dispersive X-ray (EDX) technique while acetone extracts were used for phytochemical variation using Gas chromatography-mass spectrometry (GC-MS). The result of the SEM-EDX analysis revealed that in cracked fruit deficiency of calcium, magnesium, cobalt, and tin and excess amount of chloride and potassium was found when compared with healthy fruit while lupeol acetate and sclareol phytochemical were observed in healthy fruit while cracked fruit showed its absence.

Keywords: Elemental analysis, GC-MS, Phytochemical constituents, *Punica granatum* L

Asian Pac. J. Health Sci., (2022); DOI: 10.21276/apjhs.2022.9.4S1.10

Introduction

Pomegranate (*Punica granatum* L.) is economically vital fruit plant belonging to Punicaceae family. It is indigenous to Iran and suitable to grow in arid and semi-arid regions.[1] In medical field, pomegranate and its various parts have greater importance and have many applications.[2]

Fruit cracking is one of the serious problems that badly affect the production and quality of pomegranate.[3] The market cost then falls down and regularly instances turns into unhealthy for human intake.[4,5] Cracking of pomegranate fruit is a typical hassle over its developing areas and among all types worldwide, but the magnitude of the trouble is relying upon weather, heredity, variety, fruit growth, and cultural practice.[6]

Deficiency of nutrients such as copper, zinc, calcium, potassium, and manganese results in development of cracking as they are involved in several physiological activities.[7] It has been found that solutions of calcium can help in reducing cracking in pomegranate as calcium plays an important role in maintaining adsorption of water by plant roots.[8] Borax was found effective in reducing fruit cracking and expanding yield of pomegranate plants.[9] Growth regulators such as gibberalic acid and micronutrients showed beneficial effect against cracking of pomegranate fruit in cultivar Jodhpur red.[10]

Phytochemicals are naturally occurring chemicals that are present in various medicinal plants, leaves, vegetables, and roots that have protecting properties against several diseases. Phytochemicals are of two types primary and secondary metabolite. Primary metabolite contains chlorophyll, proteins, and common sugars while secondary metabolite contains terpenoid, alkaloids, and phenolic compounds.[11] The main aim of the study is to investigate elemental composition in leaves and phytochemical variation in plant bearing healthy and cracked fruit of *P granatum L*.

Department of Chemistry, KSKV Kachchh University, Bhuj, Gujarat, India.

Corresponding Author: Pooja Mesurani, Department of Chemistry, KSKV Kachchh University, Bhuj, Gujarat, India. E-mail: mespooja2210@gmail.com

How to cite this article: Mesurani P, Ram VR. Elemental and Phytochemical Variation among Healthy and Cracked Fruit of *Punica granatum* L. From Selected Areas of Kachchh Region, India. Asian Pac. J. Health Sci., 2022;9(4S1):67-71.

Source of support: None

Conflicts of interest: None.

Received: 12/5/22 **Revised:** 22/6/22 **Accepted:** 12/7/22

Materials and Methods

Leaves of *P granatum L*. were collected from village Lohariya Mota of Anjar Town of Kachchh district in month end of January 2021. The geographical location lies at 23°09′N latitude and 69°86′E longitude. The experimental area falls under arid climate and average annual rainfall received is 412 mm. The temperature varies from 44°C to 50°C in summer season and during winter season temperature drops. The soils were slightly alkaline and non-saline in nature (pH 7.85 and EC 1.07 dS m-1) and contained 1.68% organic carbon.

Two different orchards of pomegranate were selected, one orchard containing plants with healthy fruits and another containing plant with almost all cracked fruits. Both orchards were 1.5 km apart. The plants were 2 1/2 years old and cultivated under drip irrigation systems.

Fresh green leaves all-a-round the plant were collected from the selected plants. (plant bearing healthy and cracked fruits). The collected leaves samples were air dried, powdered using laboratory sample grinder, and used for further analysis.

Elemental analysis was performed at Deparment of Chemistry, KSKV Kachchh University. Powdered sample of both leaves and soil with no further treatment was mounted on sample holder, sample

©2022 The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

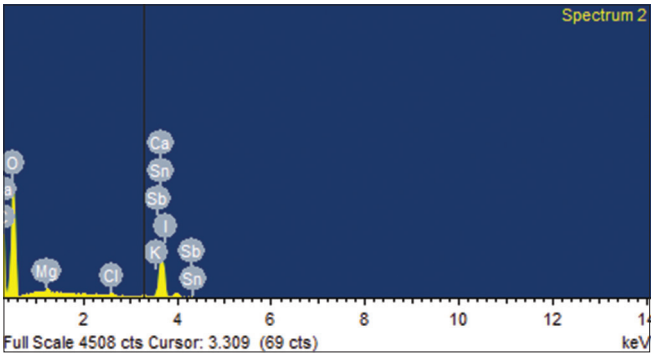

Figure 1: EDX spectrum of the leaves of pomegranate of the healthy fruit

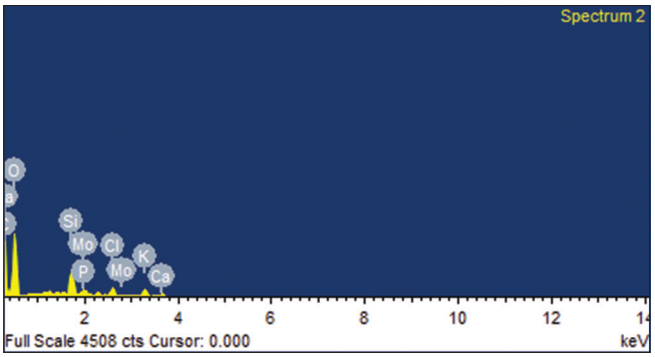

Figure 2: EDX spectrum of the leaves of pomegranate of cracked fruit

images were obtained using scanning electron microscope (JEOL JSM-6510LV) operated at 10 kV and elemental composition was determined using energy dispersive X-ray (EDX) spectrometer (Oxford) equipped with microscope and identification of phytochemicals was carried out using acetone extract, for this purpose, required quantity of leaves powder of both varieties was dipped in acetone solvent by macreation for 24 h then filtered using Whatman No. 41 and filtrate was used for the analysis, identification of this phytochemicals was carried out using gas chromatography-mass spectrometry (GC-MS) by employing 2 µL sample and peaks obtained were compared with Wiley compound library.

Phytochemical analysis for acetone extract was carried out using shimadzu QP 2010 GC consisting of auto sampler and hyphenated with mass spectrometer. For GC-MS detection, BPX (Cyanopropyl Polysilphenylene-siloxane) capillary column

Table 1: Elemental composition in leaves of plant bearing healthy and cracked fruit

Leaves of plant bearing healthy fruit		*Leaves of plant bearing cracked fruit*	
Element	*Weight %*	*Element*	*Weight %*
C	46.53	C	69.11
O	25.48	O	21.35
Mg	0.35	Si	2.65
Cl	0.39	Cl	1.50
K	1.00	K	2.20
Ca	11.50	Ca	0.90
Sn	2.94	P	0.64
Sb	8.97	Mo	1.64
I	2.84	–	–

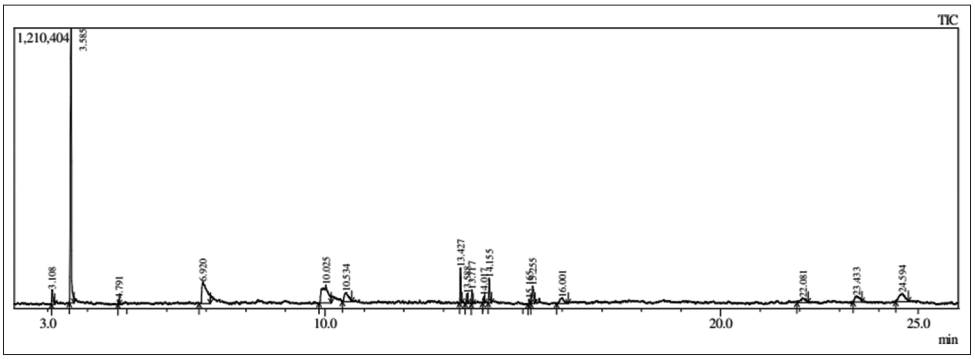

Figure 3: GC-MS chromatogram of healthy *Punica granatum* L. acetone extract

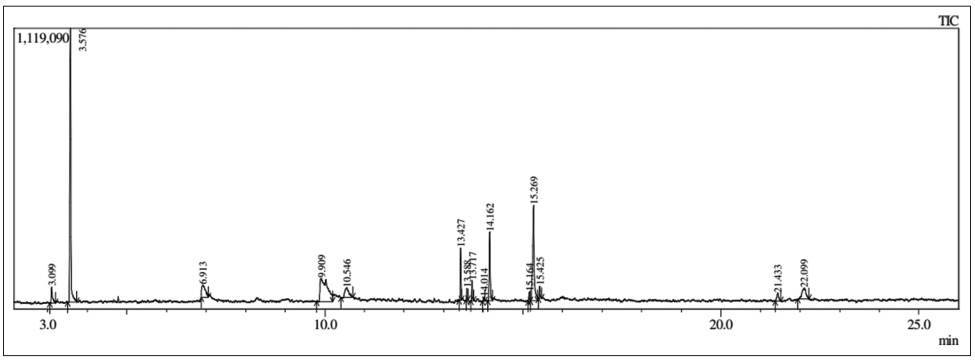

Figure 4: GC-MS chromatogram of cracked *Punica granatum* L. acetone extract

of (30 × 0.53 ID × 3 µm df) was used. For ionization in mass spectrometer, electron ionization method was used with ionization energy of 70eV. Helium gas at flow rate of 1.4 mL/min was used as carrier gas and 2 µL sample was injected in split mode for the analysis. The ion source temperature was maintained at 230˚C, injector temperature at 250˚C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 60 to 1000 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 26 min. Blank peaks were eliminated from the sample. Interpretation of the obtained peaks in spectrum was carried out using database of Wiley and obtained unknown peaks were compared with Wiley library with results indicating name, molecular weight, and structure of the components. Percentage of each component was calculated by comparing its area with total area.

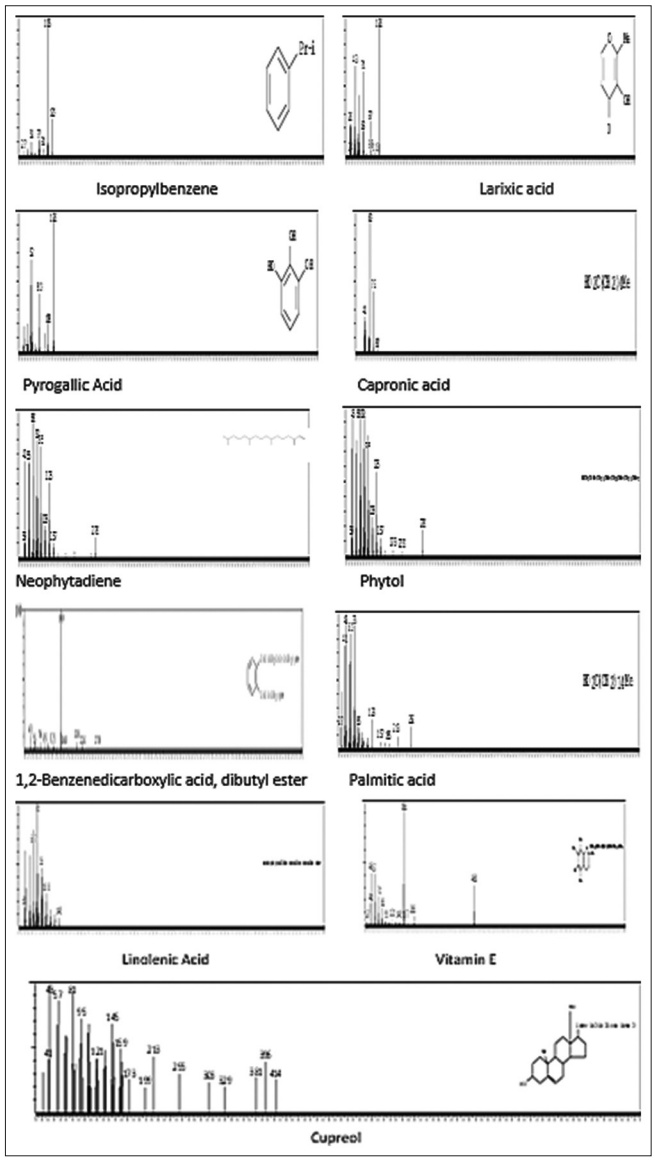

Figure 5: Mass spectrum and structure of common phytoconstituents identified by GC-MS in the acetone extracts of *Punica granatum* L. of both varieties.

Results and Discussion

Elemental Analysis

The results of EDX revealed that total nine elements were present in leaves of plant containing healthy fruits [Figure 1] while the presence of eight elements was noted in plant bearing cracked fruit [Figure 2]. From the obtained results, it was found that deficiency of Ca (0.90%), Sn and iodine was observed in leaves of cracked fruit [Table 1] According to,[12] results showed that Ca fertilization lead to 26–52% reduction in fruit cracking in evaluation with the control, with the positive influence determined with the nano-Ca fertilizer at 0.50 g Ca L-1, also four fold amount of chloride and excess potassium was found in leaves of cracked fruit [Table 1].

The high levels of potassium lead to stunted growth of plant.[13] It was found that high concentrations of Na^+ interfere with K^+ and Ca^{2+} nutrition while higher amount of Cl^- concentration reduces the photosynthetic capacity due to chlorophyll degradation.[14] Table 1 shows elemental composition in of leaves of plant bearing healthy and cracked fruit.

Phytochemical Analysis using GC-MS

GC-MS analysis of acetone extract of leaves of healthy fruit showed 14 peaks [Figure 3] indicating presence of 14 phytoconstituents, while leaves of cracked fruit showed presence of 12 peaks [Figure 4] indicating presence of 12 phytoconstituents. Mass spectra obtained were characterized and identified using Wiley library. Various phytoconstituents obtained from the leaves of healthy tree are shown in [Table 2], while present in leaves of plant bearing cracked fruit are shown in [Table 3].

Table 2: Phytoconstituents present in leaves of healthy *Punica granatum* L. by GC-MS

No.	*Retension time*	*Name of compound*	*Molecular formula*	*Molecular weight*	*Peak area %*
1	4.79	Isopropylbenzene	C_9H_{12}	120	0.44
2	6.92	Larixinic Acid	$C_6H_6O_3$	126	13.79
3	10.02	Pyrogallic acid	$C_6H_6O_3$	126	13.77
4	10.54	Capronic acid	$C_6H_{12}O_6$	116	4.61
5	13.42	Neophytadiene	$C_{20}H_{38}$	278	3.58
6	13.58	Phytol	$C_{20}H_{40}O$	296	1.16
7	14.01	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278	0.85
8	14.15	Palmitic acid	$C_{16}H_{32}O_2$	256	3.21
9	15.25	Linolenic acid	$C_{19}H_{32}O_2$	292	3.85
10	15.42	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.22
11	16.00	Vitamin E	$C_{29}H_{50}O_2$	430	2.93
12	22.08	Cupreol	$C_{29}H_{50}O$	414	2.55
13	23.43	Lupeol acetate	$C_{32}H_{52}O_2$	468	3.41
14	24.59	Sclareol	$C_{20}H_{36}O_2$	308	5.78

GC-MS: Gas chromatography-mass spectrometry

Table 3: Phytoconstituents present in leaves of cracked *Punica granatum* L. by GC-MS

No.	*Retension time*	*Name of compound*	*Molecular formula*	*Molecular weight*	*Peak area %*
1	4.79	Isopropylbenzene	C_9H_{12}	120	0.49
2	6.91	Larixic Acid	$C_6H_6O_3$	126	4.97
3	9.90	Pyrogallic acid	$C_6H_6O_3$	126	18.64
4	10.54	Capronic acid	$C_6H_{12}O_6$	116	3.97
5	13.42	Neophytadiene	$C_{20}H_{38}$	278	4.44
6	13.58	Phytol	$C_{20}H_{40}O$	296	0.90
7	14.01	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278	0.44
8	14.16	Palmitic acid	$C_{16}H_{32}O_2$	256	7.59
9	15.26	Linolenic acid	$C_{19}H_{32}O_2$	292	16
10	15.42	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.22
11	21.43	Squalene	$C_{29}H_{50}O$	414	1.67
12	22.09	Cupreol	$C_{29}H_{50}O$	414	4.79

GC-MS: Gas chromatography-mass spectrometry

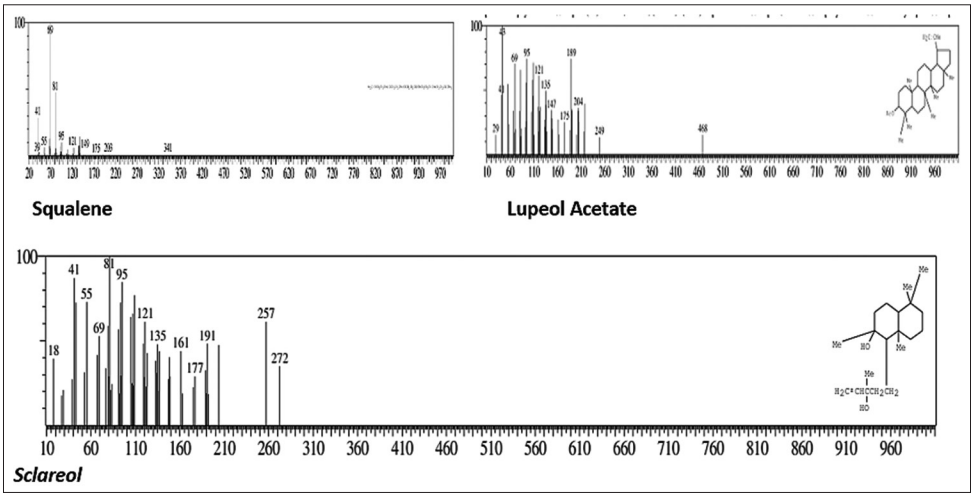

Figure 6: Mass spectrum and structure of uncommon phytoconstituents identified by GC-MS in the acetone extracts of *Punica granatum* L. of both varieties

Eleven phytoconstituents were found common in both healthy and cracked fruit. Lupeol acetate and Sclareol were additionally found in leaves of plant bearing healthy fruit while cracked fruit varied by presence of squalene [Figure 6]. Highest percentage of larixinic acid was found in healthy fruit while pyrogallic acid in cracked fruit. Figure 5 shows structure and mass spectrum of 11 phytoconstituents present in common in leaves of both the varieties of pomegranate.

Conclusion

From above EDX analysis of leaves, deficiency of Ca, I, and Sn and excess amount of potassium and chloride may be responsible for cracking of pomegranate, as higher amount of chloride and potassium leads damage to plant. Further studies would be necessary to optimize concentration studies that would be necessary to optimize concentration of Ca, Sn, I,K,Cl, and their timing of application and effect of Sb concentration in plants to reduce cracking of pomegranate which adversely affect production and quality, in addition to that sclareol and lupeol acetate were found in healthy fruit, so correlation of this two phytochemical with cracking of pomegranate should be identified.

Acknowledgment

The author thank Department of Chemistry, KSKV Kachchh University for providing necessary laboratory facilities and Council of Scientific and Industrial Research (CSIR) for providing financial support. The authors also thank farmers for providing necessary samples to carry out the work.

Copyright and Permission Statement

We confirm that the materials included in this chapter do not violate copyright laws. Where relevant, appropriate permissions have been obtained from the original copyright holder (s). All original sources have been appropriately acknowledged.

References

1. Sarkhosh A, Zamani Z, Fatahi R, Ebadi A. RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. Sci Hortic 2006;111:24-9.
2. Lansky EP, Newman RA. *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 2007;109:177-206.
3. Singh A, Shukla AK, Meghwal PR. Fruit cracking in pomegranate: Extent, cause, and management a review. Int J Fruit Sci 2020;20:S1234-53.
4. Panwar S, Desai OT, Choudhary SM. Effect of pruning on physiological disorders in pomegranates. Annal Arid Zone 1994;33:83-4.
5. Singh SP. Pomegranate in Commercial Fruits. Ludhiana: Kalyani Publisher; 1995.
6. Saad FA, Shaheen MA, Tawfik HA. Anatomical study of cracking in pomegranate fruit. J Agric Res 1988;33:155-66.
7. Sheikh M, Manjula N. Effect of chemicals on control of fruit cracking

in pomegranate (*Punica granatum* L.). Pomegranate and minor Mediterranean fruits. Adana (Turkey) 2006;35:16-9.
8. Sharma N, Belsare C. Effect of plant bio regulators and nutrients on fruit cracking and quality in pomegranate (*Punica granatum* L.). Acta Hortic 2011;890:347-52.
9. Soni AK, Gupta NK, Paliw R. Response of borax with irrigation at different intervals on fruit cracking and yield of pomegranate. Ann Arid Zone 2000;39:1256-128.
10. Kuldeep K, Joon MS, Sihog RP. Effect of micronutrients and growth regulators on premature and mature cracking of pomegranate. Hortic Sci 2001;30:207-8.
11. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev 2007;1:97-104.
12. Davarpanah S, Tehranifar A, Abadia J, Val J, Davarynejad G, Aran M, *et al*. Foliar calcium fertilization reduces fruit cracking in pomegranate (*Punica granatum* cv. Ardestani). Sci Hortic 2018;230:86-91.
13. Dhillon W, Gill PP, Singh N. Effect of nitrogen, phosphorus and potassium fertilization on growth, yield and quality of pomegranate "Kandhari". Acta Hortic 2011;890:327-32.
14. Tavakkoli E, Rengasamy P, McDonald GK. High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J Exp Bot 2010;61:4449-59.