The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2023

Volume 10, Pages 22-26

ICMeHeLS 2023: International Conference on Medical, Health and Life Sciences

Bioactive Component Analysis of Dices of Pomegranate Fruits in Different Phenotype

Sibel Bayil-Oguzkan
Gaziantep University

Elif Sine Duvenci
Duzce University

Halil Ibrahim Kilic
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract: Pomegranate fruit is a type of fruit belonging to the family of Lythraceae, consisting of shell, kernel, grains and dice. In this study, pomegranates of 3 different genotypes (local name: deve dişi, hicaz and nuz ekşisi) harvested (October-December), which are widely cultivated in the province of Gaziantep, Oğuzeli, were collected, and after identification, the dice sections were removed and dried in the shade. Total antioxidant and total oxidant levels, animicrobial activities, DNA protective activity and total flavanoid and total phenolic content were determined in 18 extracts in 3 different solvents (methanol, ethanol and DMSO) with solid-liquid extraction. Kirby-Bauer disc diffusion method was used to determine antimicrobial properties. According to the results obtained, 100% of Escherichia coli, Stenotrophomonas maltophilia, Staphylococcus aureus strains were resistant to all membrane extracts. While antioxidant levels of all membrane extracts were found highly and oxidant levels were found to be low. It was determined that the extracts have the potential to protect DNA against oxidative damage caused by UV and H_2O_2. Total phenolic determination by Folin-Ciocalteu method; Total flavonoid amount was measured by $AlCl_3$ using colorimetric method. Compared to the solvent and extraction methods used, high results were obtained in both phenolic and flavonoid determinations in all extracts, although they differed slightly among themselves. While the pomegranate fruit is consumed as food, the dice and shell parts are discarded. According to our study results, it is thought that alternative new products that can be used in complementary medicine can be obtained from pomegranate membranes that have a rich bioactive composition.

Keywords: DNA, UV, Pomegranate, Phenolic, Flavonoid

Introduction

Pomegranate is a type of fruit from the cinnagi family, consisting of hundreds of particles that form the fruit body with small seeds, with a slightly sour and sweet taste depending on the type, grown in temperate climates. Pomegranate basically consists of 4 main parts. These parts are; It consists of shell, kernel, grains, and white membrane. Pomegranate consists of 60-67% grains and 33-40% peel. The juice of pomegranate fruit is also made from 76-85% fruit grains and 45-61% whole fruit. Pomegranate fruit consists of 75% moisture, 1.6% protein, ascorbic acid 16 mg/1000 g, ash 0.7%, 0.58% acidity and high amount of minerals. The chemical composition of the pomegranate fruit varies according to the variety, growing region, climate, ripening, planting application and storage conditions (Jurenka, 2008) (Pooja et al., 2017). The pomegranate tree is a deciduous tree

and has spread to regions similar to the Mediterranean climate, which is the most suitable climate for the cultivation of superior pomegranates. Pomegranate is a temperate climate plant and requires high temperature levels to reach proper maturity. The maturity index is the ratio of soluble dry matter to total acidity, and this index is used to classify pomegranates as sweet, tart and negative. A maturity index of 31-98 is grouped as sweet, 17-24 as mayhos and 5-7 as sour species (Martinez et al., 2006).

The worldwide increase in interest in pomegranate and pomegranate-based products is not only related to the taste of the fruit, but there are many studies in scientific studies that prevent low-intensity and high-intensity cholesterol oxidation, lower blood pressure and reduce the development of vascular occlusion. In the emergence of these beneficial effects of pomegranate-based products, reference is made to phenolic compounds with antioxidant properties (Çam et al., 2009). Data from epidemiological studies show that consumption of fruits and vegetables high in phenolic compounds reduces mortality from cardiovascular, cerebrovascular diseases and cancer. Phenolic compounds show their beneficial effects by destroying free radicals. In recent years, the trend towards fruit juices such as grape juice and blueberry juice, which are high in phenolic antioxidants, has been increasing. Pomegranate juice is a popular fruit juice with its phenolic compounds and important biological activities (Husari et al., 2014; DiMarco-Crook et al., 2015).

The effects of pomegranate peel and seeds on health have been evaluated in different studies in the literature, but no study on pomegranate membranes has been found. Therefore, in this study, some bioactive component analyzes were studied in different extractions of pomegranate membranes.

Method

Antimicrobial Analysis

Kirby-Bauer disk diffusion method was used to determine the antimicrobial properties of 18 samples. The isolates were inoculated on MH agar medium using the spread method. This process was carried out in two stages. The density of the strains was adjusted according to the 0.5 McFarland standard and sowing was done. Paper discs containing 20 µl of plant extracts were placed on the surface of the petri dishes, which were inoculated immediately afterward, with the help of sterile forceps, and left for 24 hours of aerobic incubation at 35 °C. During this process, 22 mm between the discs and 14 mm from the edge of the petri dish so that the zones to be formed do not overlap each other. The distance was taken into account. Zone diameters were measured at the end of incubation. The results showed bacterial strains according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) document Version 10 criteria; assessed as resistant and susceptible. According to the results obtained, 100% of Escherichia coli, Stenotrophomonas maltophilia, Staphylococcus aureus strains showed resistance to all plant extract samples.

DNA Protective Activity

PBR322 plasmid DNA isolated from Escherichiacoli was used in the study to determine the DNA protective activity with pomegranate extracts created with ethanol, methanol and DMSO. The density of the gel used in the study was prepared according to the number of base pairs in the relevant DNA. With this method, it is aimed to determine whether the extracts we have created in the presence of UV rays and H_2O_2, which cause damage to DNA, have the potential to prevent DNA damage.

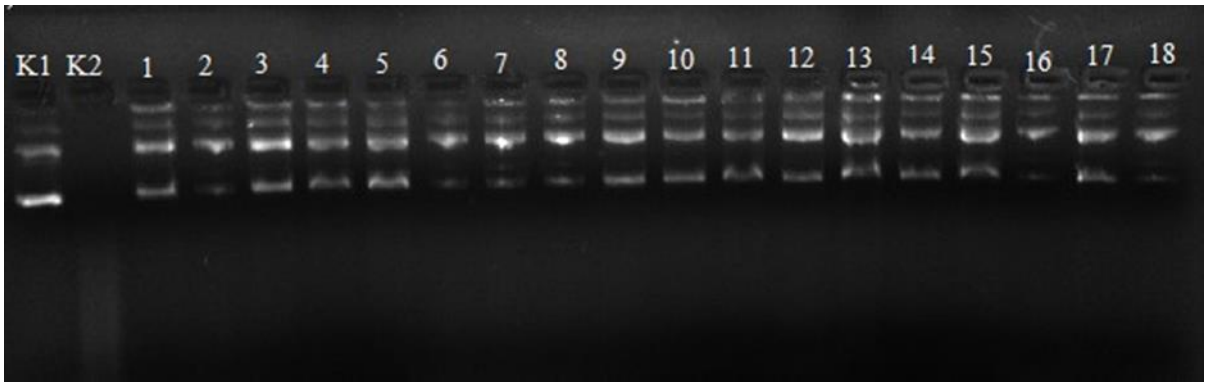

Figure 1. DNA protective activities band

It has been determined that the pomegranate extracts, especially the samples numbered 1,3,4,5,9,10,11,12,13,14,15 and 17, have a protective effect potential against oxidative damage caused by UV and H_2O_2. Therefore, it is thought that alternative new products can be produced by determining these compounds in pomegranate, which has a very rich bioactive composition. It is thought that the data obtained from this study have the potential to provide both ideas and data to new studies by creating data for researchers working in this field.

K1: Plazmit DNA (3 µl) + dH_2O (6 µl)
K2: Control: Plazmit DNA (3 µl) + dH_2O (6 µl)+ UV+ H_2O_2 (1 µl)

1. Plazmit DNA (3 µl) + NE Methanol Ultrasonic (4 hours) (5µl)+ UV+ H_2O_2 (1 µl)
2. Plazmit DNA (3 µl) + NE Ethanol (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
3. Plazmit DNA (3 µl) + NE Methanol (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
4. Plazmit DNA (3 µl) + NE DMSO Ultrasonic (4 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
5. Plazmit DNA (3 µl) + NE DMSO (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
6. Plazmit DNA (3 µl) + NE Ethanol Ultrasonic (4 hours) (5 µl) + UV+ H_2O_2 (1 µl)
7. Plazmit DNA (3 µl) + HN Ethanol Ultrasonic (4 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
8. Plazmit DNA (3 µl) + HN Ethanol (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
9. Plazmit DNA (3 µl) + HN Methanol (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
10. Plazmit DNA (3 µl) + HN Methanol Ultrasonic (4 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
11. Plazmit DNA (3 µl) + HN DMSO Ultrasonic (4 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
12. Plazmit DNA (3 µl) + HN DMSO (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
13. Plazmit DNA (3 µl) + DD DMSO Ultrasonic (4 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
14. Plazmit DNA (3 µl) + DD Methanol Ultrasonic (4 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
15. Plazmit DNA (3 µl) + DD DMSO (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
16. Plazmit DNA (3 µl) + DD Ethanol (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
17. Plazmit DNA (3 µl) + DD Methanol (16 hours) (5 µl)+ UV+ H_2O_2 (1 µl)
18. Plazmit DNA (3 µl) + DD Ethanol Ultrasonic (4 hours) (5 µl)+ UV+ H_2O_2 (1 µl)

Total Antioxidant and Total Oxidant Levels

Commercially available kits were used to determine the antioxidant capacity of pomegranate membrane extracts. Antioxidant substances in the sample convert the radical to the dark blue-green colored ABTS (2,2 AzinoBis (3-Ethyl Benzo Thiazoline-6- Sulfonic Acid) form of reduced ABTS, and the change in absorbance at 660 nm indicates the total antioxidant level of the sample. The synthetic antioxidant and Vitamin E analogue Trolox was used as a positive control (Halliwell et al., 1994). Rel Assay Diagnostics-TAS Assay Kit was used in this study. Total oxidant level determination test is calibrated with hydrogen peroxide. Ferrous ion in the presence of oxidant Oxidizes the chelator complex to ferric ion. The oxidation reaction intensity increases due to the presence of oxidants in the reaction medium. Ferric ion forms a colored complex in acidic medium. Color density is determined spectrophotometrically.

Table 1. Result of TAS

TAS Results mmol/L						
Sample No	1	2	3	4	5	6
	4,2322	4,2022	4,2285	4,2322	4,2472	4,191
Sample No	7	8	9	10	11	12
	4,1948	4,1873	4,2097	4,1873	4,1798	4,2509
Sample No	13	14	15	16	17	18
	4,2022	4,206	4,2285	4,1835	4,1723	4,1798

Table 2. Result of TOS

TOS Results mmol/L						
Sample No	1	2	3	4	5	6
	0,0273	0,0112	0,0169	0,0162	0,0069	0,0214
Sample No	7	8	9	10	11	12
	0,0064	0,0108	0,0233	0,0323	0,0564	0,1054
Sample No	13	14	15	16	17	18
	0,0114	0,0343	0,0179	0,0155	0,0137	0,0156

Mesurement of Total Phenolic Substance and Total Flavonoid Substance Amount

In the liquid extracts obtained by trying different extraction methods, the total amount of phenolic substance as gallic acid equivalent. The determination of the total flavonoid substance amount was measured as the equivalent of quercetin by UV-VIS spectrophotometer and photometric method. Total phenolic content was determined by the Folin-Ciocalteu method. Gallic acid was used as a standard and readings were taken at 760 nm. 0.5 N Folin reagent was prepared to be used in the experiment and 10% Na_2CO_3 was used as a color indicator. The stock standard was prepared at 1000 ppm with methanol and other standards were prepared by serial dilution. A calibration chart specific to the gallic acid standard was drawn and the total phenolic acid concentrations were determined with the absorbances obtained from the UV readings of the liquid extracts. Total flavonoid substance determination was made with $AlCl_3$, which is a colorimetric method. Quercetin was used as a standard and readings were made at 415 nm. A 2% $AlCl_3$ solution was prepared to be used in the experiment. The stock standard was prepared at 1000 ppm with methanol and other standards were prepared by serial dilution. A calibration chart specific to the quercetin standard was drawn and total flavonoid acid concentrations were determined by absorbances obtained from UV readings of liquid extracts.

Table 3. Total Phenolic Substance Results (mg\L)

Phenolic Substance Results (mg\L)						
Sample No	1	2	3	4	5	6
	6485,95	9137,97	6351,42	7679,43	5144,58	5276,38
Sample No	7	8	9	10	11	12
	3706,66	3507,8	4102,06	7057,79	4423,99	7127,8
Sample No	13	14	15	16	17	18
	4591,69	7043,05	11724,3	8581,92	7912,29	4336,3

Table 4. Total Flavonoid Substance Results (mg\L)

Flavonoid Substance Results (mg\L)						
Sample No	1	2	3	4	5	6
	495,98	2319,8	1148,55	1203,83	1647,7	1387,0
Sample No	7	8	9	10	11	12
	660,66	636,36	1462,01	1226,13	726,42	936,62
Sample No	13	14	15	16	17	18
	1248,83	1136,76	2080,32	2425,99	1297,01	1103,28

When the amounts of phenolic and flavonoid substances in the extracts prepared by co-solvent and co-extraction of three different pomegranate membranes were compared. In the extracts prepared with dimethylsulfoxide solvent and ultrasonic assisted extraction, the best phenolic substance results were obtained while the extracts prepared from the pulps of the muzzle were obtained in the classical extraction. When the amounts of flavonoid substances were examined, it was observed that the hijaz pomegranate membrane was high in ultrasonic assisted extraction. In the classical extraction, however, the nuz ekşi was found to be high.

Conclusion

In the pomegranate plant in Oguzeli, which is a priority in terms of rural development, only 45-50% of the fruit can be utilized in total by making only the juice of the pomegranate fruits or boiling the water and making the pomegranate syrup. The peel and seeds remaining during processing into pomegranate juice are industrially valuable waste products. Despite the active ingredient content, the peel, core and membrane parts (dice) are unfortunately in the category of waste materials due to the limited conditions and knowledge of the farmers. In our current study, it was determined that pomegranate membranes which are waste materials, showed good antioxidant properties and were UV protective against DNA. As a continuation of this rural development, we believe that the determination of the pharmacological bioactive components of the waste materials in the existing pomegranate processing plants and the evaluation of the materials released after the processing of the water will have an effective effect on the development in this area.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

This article was presented as an oral presentation at the International Conference on Medical, Health and Life Sciences (www.icmehels.net) held in Budapest/Hungary on July 06-09, 2023.

References

Çam, M., Hışıl, Y., & Durmaz, G. (2009). Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chemistry, 112*(3), 721–726.

DiMarco-Crook, C., & Xiao, H. (2015). Diet-based strategies for cancer chemoprevention: The role of combination regimens using dietary bioactive components. *Annual Review of Food Science and Technology, 6*(1), 505–526.

Halliwell, B. (1994). Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? *The Lancet, 344*(8924), 721–724.

Henry, W. A., III. (1990, April 9). Making the grade in today's schools. *Time, 135*, 28-31.

Husari, A., Khayat, A., Bitar, H., Hashem, Y., Rizkallah, A., Zaatari, G., & El Sabban, M. (2014). Antioxidant activity of pomegranate juice reduces acute lung injury secondary to hyperoxia in an animal model. *BMC Research Notes, 7*(1), 664.

Jurenka, J.S. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Alternative Med. Rev., 13,* 128–144.

Martínez, J. J., Melgarejo, P., Hernández, Fa., Salazar, D. M., & Martínez, R. (2006). Seed characterisation of five new pomegranate (Punica granatum L.) varieties. *Scientia Horticulturae, 110*(3), 241–246.

Pooja, S., Sarah, F. M. & Farrukh A. (2017). Pomegranate for prevention and treatment of cancer: An update. *Molecules, 22*(1), 177.

Author Information

Sibel Bayil-Oguzkan
Gaziantep University
Gaziantep, Turkey
Contact e-mail: *bayil@gantep.com*

Elif Sine Duvenci
Duzce University
Düzce, Turkey

Halil Ibrahim Kilic
Gaziantep University
Gaziantep,Turkey

Mehmet Ozaslan
Gaziantep University
Gaziantep,Turkey

To cite this article:

Bayil-Oguzkan, S., Duvenci, E.S., Kilic, H.I., & Ozaslan, M. (2023). Bioactive component analysis of dices of pomegranate fruits in different phenotype. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 10,* 22-26.