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Kinetics on the DPPH· reaction with hydroalcoholic extracts from various pomegranate parts

Gabriela Sicoe[1], Claudia Izabela Oprinescu[2], Giulia Mădălina Golea[2], Adrian Riviş[2], Nicoleta G. Hădărugă[2*]

[1]"Mihai Viteazul" Technical School, 410191-Oradea, Poieniţei 25, Romania
Department of Food Science,
[2]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" - Timişoara, 300645-Timişora, Aradului Str. 119, Romania

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Abstract

In the present study the antioxidant activity and kinetics on DPPH· (2,2-diphenyl-1-pycryl-hydrazyl) reaction in the presence of hydroalcoholic extracts from various pomegranate parts have been investigated. Pomegranate, the fruit of the small three *Punica granatum* L., is extensively used for obtaining food products, especially juices and alcoholic drinks, also having curative properties due to the presence of polyphenolic compounds and vitamin C.

Pomegranate extracts were obtained from various fruit parts such as red or white internal shell, pulp, seeds and whole fruit, using ethanol 96% as solvent or the raw juice, at room temperature. The kinetic of the DPPH· reaction in the presence of pomegranate extracts (diluted at 1:50 and 1:100) was determined spectrophotometrically, measuring the variation of the absorbance at 518 nm during fifteen minutes. Consequently, the variation of DPPH· concentration during the reaction was determined using a calibration curve for the free radical. The mean DPPH· reaction rates were obtained for the pseudolinear intervals of 0-30 s, 30-90 s and 90-900 s. The mean DPPH· reaction rate for the first interval had values in a wide range of 0.17-3.03 μM/s, with maximum values for pomegranate red shell and pulp extracts and the lowest ones for seeds extracts. On the other hand, the corresponding values for the second interval were in a narrow range of 0.05-0.6 μM/s, with the same observation for the lowest values for pomegranate seeds extracts. The antioxidant activity of these extracts were significantly higher for the pomegranate red and white internal shell extracts, as well as for the pulp extracts (46.9-54.9%), in comparison with the pomegranate juice samples (7.9-18.4%) and only 3.9-5.1% for the seeds extracts, after 1½ minutes of evaluation by DPPH· method. This study demonstrates the value of the pomegranate red and internal white shell (generally considered as waste) from the antioxidant activity point of view and suggest the recovery and re-utilization of such by-products in the food, pharmaceutical or cosmetic fields.

Keywords: pomegranate extracts, antioxidant activity, DPPH·, kinetics, polyphenols

1. Introduction

Pomegranate (*Punica granatum* L., Punicaceae) is a small tree originates from Iran and grows in whole Asia, in South Europe, in North and South of America as well as in South Africa [1,2]. The fruit of *P. garnatum* L. have nutritional and medicinal values, while the tree is important for its ornamental characteristics [3]. Pomegranate has many biological activities, being used in traditional medicine from ancient times. It was used against inflammations, diarrhea, or intestinal worms. Various studies for its therapeutic potential such as

Corresponding author: *e-mail:* nico_hadaruga@yahoo.com

antimicrobial, anti-allergic, anti-influenza and anti-malarial, cancer chemoprevention, anti-ulcer and antidiabetic, have been performed [4,5].

There is a wide distribution of compounds in pomegranate [6,7]. The main classes identified in this fruit are simple sugars, aliphatic organic acids (e.g., citric, malic, tartaric, fumaric, and succinic acids), as well as phenolic or enolic acids having antioxidant properties. It is the case of enolic furanolactones (ascorbic acid or vitamin C), hydroxybenzoic acids (gallic, ellagic, 3,3'-di-*O*-methylellagic, and 3,3',4'-tri-*O*-methylellagic acids), hydroxycinnamic acids or phenylpropanoids (caffeic, chlorogenic, and *p*-coumaric acids), and cyclitol carboxylic acids (e.g., brevifolin carboxylic acid 10-monopotassium sulphate). Important organic compound classes from pomegranate having antioxidant properties are flavonoids, flavonoid glycosides, anthocyanidins and anthocyanins. The most important compounds from these classes are catechin, epicatechin, epigallocatechin 3-gallate, quercetin, kaempferol, its glucoside and rhamnoglucoside, rutin, luteolin, apigenin and their glucosides, and naringenin, from the flavonoid derivatives, as well as delphinidin, cyanidin, pelargonidin and their glucosides and di-glucosides from the anthocyanidin and anthocyanin classes. Other organic compounds identified in various parts of pomegranate are ellagitannins (e.g., punicalin, punicalagin, corilagin, casuarinin, gallagyldilacton, pedunculagin, tellimagrandin, granatins A and B, punicafolin, punicacortein A and B), amino acids (proline, valine, methionine), indoleamines (tryptamine, serotonine, melatonine), alkaloids, especially in bark and root (pelletierine and derivatives, sedridine, hygrine), fatty acids in seeds (palmitic, stearic, oleic, linoleic, and punicic acids), sterols in seeds (β-sitosterol, stigmasterol, camesterol, or daucosterol), triterpenoids in pomegranate flower and seeds (ursolic, oleanolic, maslinic, and asiatic acids), glycolipids such as cerebroside, and phenyl aliphatic glycosides in seeds (e.g., phenethyl rutinoside or icariside D1). Some representative compounds identified in pomegranate are presented in Figures 1-3.

Antioxidant activity is one of the most important property of pomegranate. Biological activities of pomegranate is closely linked to the presence of organic compounds having antioxidant characteristics (i.e. phenolic and polyphenolic compounds) [8-11].

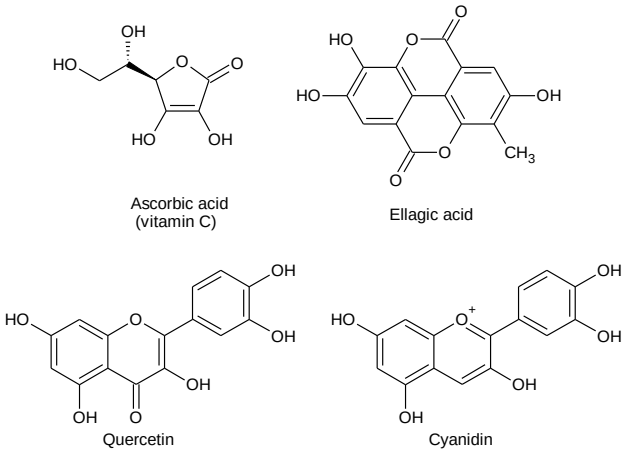


Figure 1. Representative compounds from pomegranate fruit having antioxidant activity

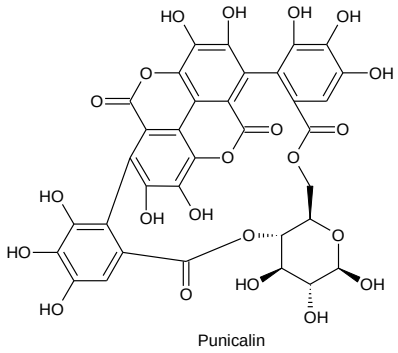


Figure 2. Structure of punicalin (ellagitannin class) from bark and root of pomegranate

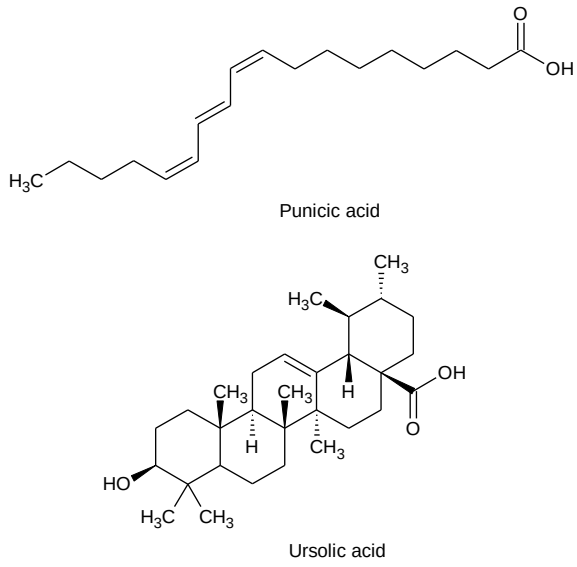


Figure 3. Representative structures from pomegranate seeds

In the present study the overall antioxidant activity by DPPH· method and the kinetic of the DPPH· reaction in the presence of pomegranate extracts obtained from various fruit parts have been investigated. The goal was to evidence the important parts of pomegranate, especially waste or by-products from juice industry, from the antioxidant activity point of view for re-utilization and possible application in food, pharmaceutical or cosmetic fields.

2. Materials and Method

2.1. Pomegranate samples and preparation of juices and hydroalcoholic extracts

Pomegranate fruits were purchased from the local market (Timişoara, Romania) and were from the *P. granatum* L. species, harvested from the Mediterranean Europe in the spring of 2017. Fruits were squeezed using a home squeezer. On the other hand, different parts of pomegranate were carefully separated, weighted, sealed and stored in the refrigerator up to one day until extractions. Pomegranate fruit samples had a mean weight of 263.6 (± 33.8) g, while the fruit parts had the following masses: 67.6 (± 14.3) g of pomegranate juice (abbreviated as "*J*"), 158.1 (± 28.5) g for the pomegranate shell (both red and white internal shell parts, abbreviated as "*RS*" and "*WS*", respectively), 8.4 (± 1.0) g for the pulp collected from the closer part of the seeds (abbreviated as "*P*"), and 32.3 (± 9.0) g of pomegranate seeds (abbreviated as "*S*").

Samples of *RS*, *WS*, *P*, and *S*, of the corresponding masses of 10.0 (± 0.1), 10.0 (± 0.1), 8.8 (± 1.1), and 10.0 (± 0.1) g, respectively, were finely grounded in a ceramic mortar and extracted with ethanol (96%, *v/v*, Chimopar, Bucharest) of 20 mL for the first three sample types and 25 mL for the pomegranate seeds. The extraction was performed in sealed flasks of 150 mL at room temperature for 2 h, under intermittent stirring. The extracts were then filtered and adjusted to 25 mL with the same solvent. Generally, extractions were carried out in triplicate.

2.2. Evaluation of the antioxidant activity of pomegranate juice and hydroalcoholic extracts

Pomegranate juice samples and pomegranate hydroalcoholic extracts from different fruit parts were subjected to antioxidant activity evaluation using the spectrophotometric DPPH· method (the absorbance of samples did not significantly superimposed on the DPPH· absorbance). Both pomegranate juice and alcoholic extracts were progressively diluted by 50 and 100 folds with ethanol for an appropriate antioxidant activity evaluation. The diluted juice and extract samples were mixed with 1 mM ethanolic DPPH· solution in ethanol (volume ratio of 1:1:4) in a 4 mL spectrophotometric cuvette (1 cm length) and the absorbance at 518 nm was recorded for 15 minutes using a CamSpec M501 single beam spectrophotometer (CamSpec Ltd., Cambridge, United Kingdom). The antioxidant activity (abbreviated as "*AO*", as percent) was determined according to the following equation:

$$AO\ (\%) = [1 - A_{t=0} / A_t] \cdot 100$$

where $A_{t=0}$ represent the absorbance of the mixture at the start of spectrophotometric monitoring (at 518 nm) and A_t represent the absorbance of the mixture at the reaction time t (s).

2.3. Kinetics of DPPH· reaction in the presence of pomegranate juice and hydroalcoholic extracts

The kinetics of DPPH· reaction with the antioxidant compounds from pomegranate juice and extract samples was estimated as the mean DPPH· reaction rates on the pseudolinear intervals from the *Concentration (DPPH·) versus Time (s)* plot. Three time intervals were identified: 0-30 s for the fast reaction of DPPH·, 30-90 s for the middle reaction of the free radical and 90-900 s (and even more) for the completion of the DPPH· reaction in the presence of antioxidant compounds. Thus, the following equations were used for kinetic study:

$$v_1 = - \Delta C_{(DPPH\cdot)1} / \Delta t_1$$

$$v_2 = - \Delta C_{(DPPH\cdot)2} / \Delta t_2$$

$$v_3 = - \Delta C_{(DPPH\cdot)3} / \Delta t_3$$

where v_1, v_2, and v_3 represents the mean DPPH· reaction rates in the presence of pomegranate juices and extracts for the first (*1*), second (*2*) and third (*3*) time interval (in μM/s), $\Delta C_{(DPPH\cdot)1\text{-}3}$ represents the variation of the DPPH· concentration (in μM) for those three time intervals, $t_{1\text{-}3}$ (s).

2.4. Statistical and correlation analysis

Analysis of variance (ANOVA) have been used for handling of extraction data, as well as for kinetic parameters, using the Microsoft Excel® 2013 program from the Microsoft Office Professional Plus 2013 package (Microsoft Corporation, USA). The mean values and standard deviations (SD) have

been determined. On the other hand, the linear correlations of DPPH· concentration and reaction time for various time intervals have been performed by root mean square (RMS) method using the Statistica 7 package (StatSoft, Inc.).

3. Results and Discussion

The yield of pomegranate juice separation was 25.9 (± 5.3) %, as reported to the whole fruit, while the overall shell (both red and white internal shell parts) was at a ratio of 60 (± 8.6) %. However, all these pomegranate fruit parts, even consumed or waste parts, have been investigated for their antioxidant capacity and possible food, pharmaceutical, cosmetic applications.

The maximum absorbance for DPPH· ethanolic solutions was recorded at 518 nm (e.g., $A_{(518\ nm)}$ = 1.436 for 200 μM DPPH· solution), while the diluted samples of pomegranate juices and extracts had almost no absorbance (Figure 4). The correlation of absorbance of DPPH· ethanolic solutions at various concentrations in the range of 25-300 μM and 518 nm reveals a DPPH· standard curve as follows:

$$A_{(518\ nm)} = 0.006259(\pm 0.00011) \cdot C_{(DPPH\cdot)}$$

where $A_{(518\ nm)}$ is the absorbance of DPPH· ethanolic solution at 518 nm, while $C_{(DPPH\cdot)}$ represents the DPPH· concentration (μM).

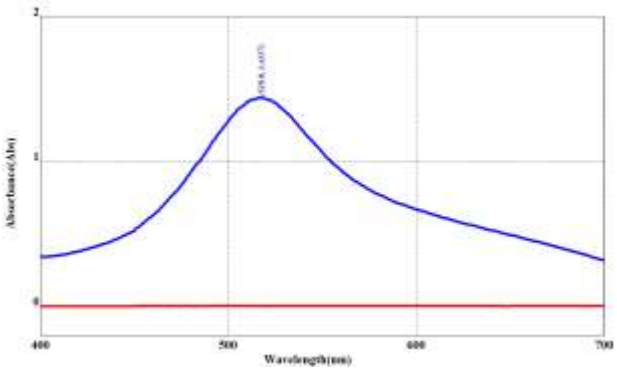


Figure 4. UV-Vis spectra for DPPH· standard solution (in ethanol at a volume ratio of 1:5, blue) and diluted pomegranate juice (1:50 in ethanol, red)

The spectrophotometric monitoring of the absorbance of pomegranate juices or hydroalcoholic extracts and DPPH· ethanolic solution (at 1:1:4 volume ratio and 518 nm) reveals an inverse logarithmic variation, more evident for 1:50 dilution samples than for 1:100 dilutions (Figures 5-9).

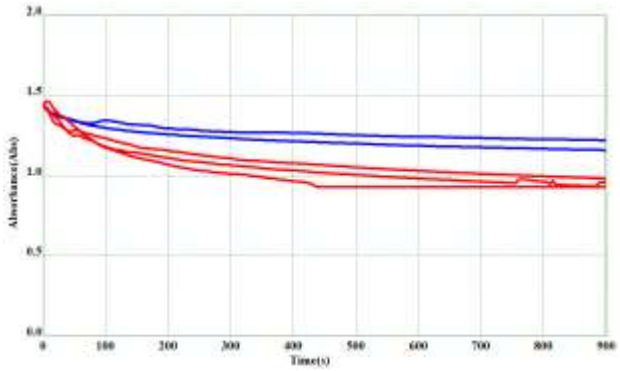


Figure 5. *Absorbance versus Time* (s) from the spectrophotometric monitoring of DPPH· reaction in the presence of pomegranate juice "*a*" (1:50 dilution as triplicate, red, and 1:100 dilution, as duplicate, blue)

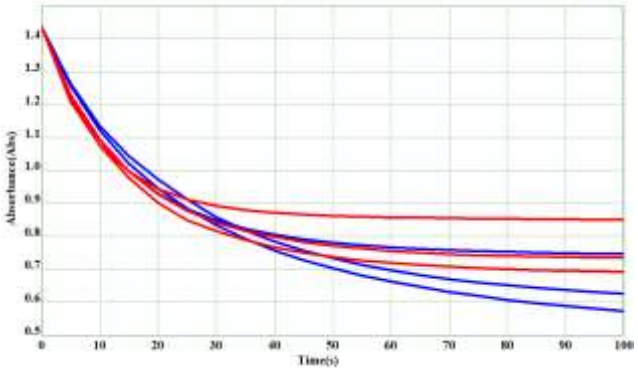


Figure 6. *Absorbance versus Time* (s) from the spectrophotometric monitoring of DPPH· reaction in the presence of pomegranate red shell ethanolic extracts (1:50 dilution, red, and 1:100 dilution, blue)

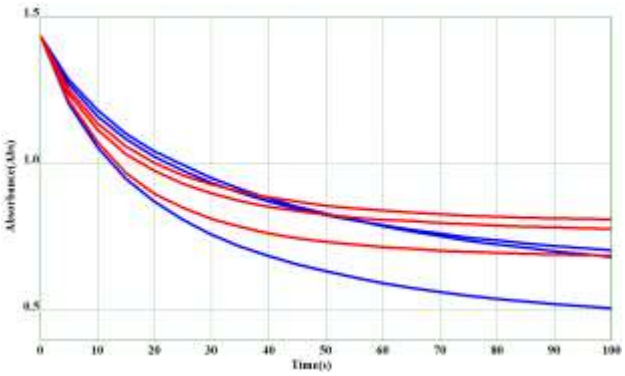


Figure 7. *Absorbance versus Time* (s) from the spectrophotometric monitoring of DPPH· reaction in the presence of pomegranate white interior shell ethanolic extracts (1:50 dilution, red, and 1:100 dilution, blue)

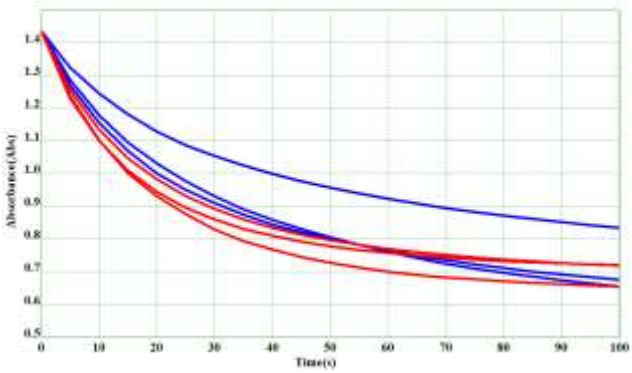


Figure 8. *Absorbance versus Time* (s) from the spectrophotometric monitoring of DPPH· reaction in the presence of pomegranate pulp ethanolic extracts (1:50 dilution, red, and 1:100 dilution, blue)

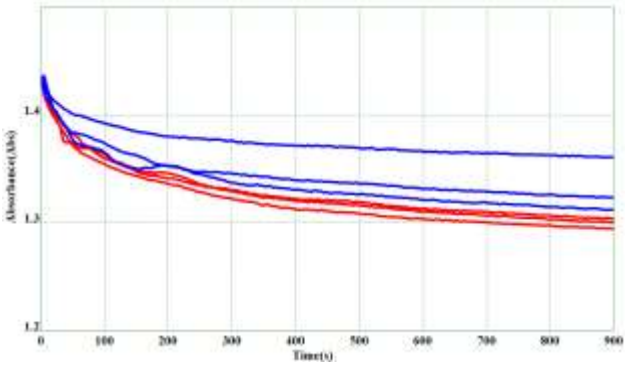


Figure 9. *Absorbance versus Time* (s) from the spectrophotometric monitoring of DPPH· reaction in the presence of pomegranate seeds ethanolic extracts (1:50 dilution, red, and 1:100 dilution, blue)

Actual antioxidant activity (*AO*, %) of pomegranate juices and hydroalcoholic extracts from various fruit parts have been determined as the difference from one hundred of the ratios (as percent) between the absorbance of the pomegranate diluted extracts : DPPH· solution : ethanol mixtures at the moment *t* and the starting moment, *t* = 0. The *AO* values clearly differs on both time and extract types (Table 1). The *AOs* of 1½ minutes are representative. Thus, the *AO* for pomegranate juices were in the range of 13.7-18.4 % for 1:50 dilutions and 7.9-8.2 % for 1:100 dilutions. There were no significant differences between those values obtained for 1:50 dilutions and especially 1:100 dilutions (only 0.3% *AO* units).

By far, the most active samples were the ethanolic extracts obtained from red and white internal shell, as well as from the pulp around the seeds. The *AO* values were in the ranges of 46.9-54.9% at 1½ minutes and 48.4-65% at the final of monitoring (15 minutes, Table 1).However, there were not significant differences between 1:50 and 1:100 dilutions or after 1½ minutes of monitoring, probably due to the limit of the quantity of DPPH· compound in the solution used for analysis (according to the methodology). These means that higher dilution is need for some of such pomegranate concentrated samples. On the other hand, the *AO* values are considerably lower for seeds extracts, where the oil is much more concentrated (3.9-5.1% at 1½ minutes and 7.2-9.5% at 15 minutes) (Table 1 and Figures 10-14).

Table 1. Antioxidant activity of pomegranate juice and extract samples at different time intervals

Nº	Code*	*AO* (90 s) (%)**	*AO* (15 min) (%)**
1	*J(1:50_a)*	15.93 ± 1.98	33.56 ± 1.80
2	*J(1:100_a)*	8.06 ± 1.58	17.21 ± 3.07
3	*J(1:50_b)*	18.42 ± 5.46	39.58 ± 4.12
4	*J(1:100_b)*	7.88 ± 4.53	17.82 ± 8.56
5	*J(1:50_c)*	13.65 ± 1.40	27.51 ± 1.80
6	*J(1:100_c)*	8.22 ± 1.49	17.94 ± 0.38
7	*RS(1:50)*	46.97 ± 5.64	48.94 ± 5.43
8	*RS(1:100)*	54.19 ± 5.75	58.38 ± 7.96
9	*WS(1:50)*	46.88 ± 4.44	48.39 ± 4.54
10	*WS(1:100)*	54.87 ± 7.62	64.99 ± 6.22
11	*P(1:50)*	50.90 ± 2.58	53.3 ± 2.97
12	*P(1:100)*	48.51 ± 6.81	59.32 ± 5.23
13	*S(1:50)*	5.12 ± 0.34	9.48 ± 0.32
14	*S(1:100)*	3.91 ± 0.91	7.22 ± 1.77

* *J(1:50/100_a/b/c)* – pomegranate juice at 1:50 or 1:100 dilution (samples "*a*", "*b*" and "*c*"); *RS(1:50/100)* – pomegranate red shell ethanolic extract at 1:50 or 1:100 dilution; *WS(1:50/100)* – pomegranate white internal shell ethanolic extract at 1:50 or 1:100 dilution; *P(1:50/100)* – pomegranate pulp ethanolic extract at 1:50 or 1:100 dilution; *S(1:50/100)* – pomegranate seeds ethanolic extract at 1:50 or 1:100 dilution;
** Antioxidant activity at 90 s and 15 minutes

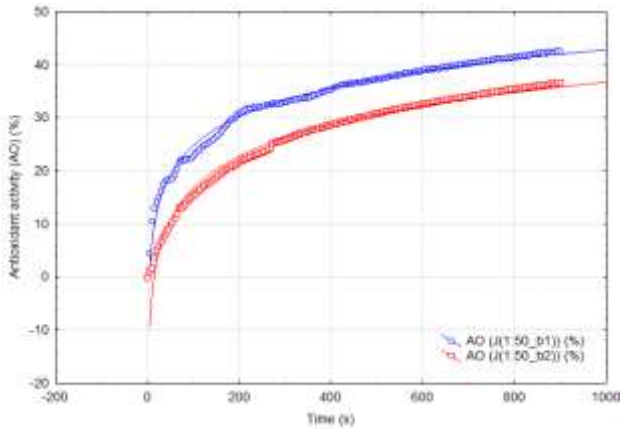


Figure 10. Variation of the antioxidant activity (*AO*, %) of the pomegranate juice (sample "*b*" at 1:50 dilution – duplicate analysis)

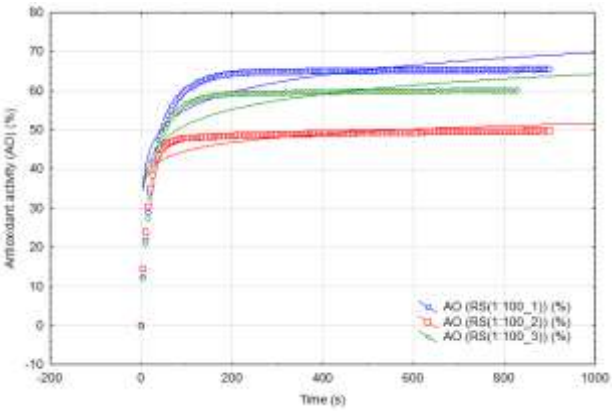


Figure 11. Variation of the antioxidant activity (*AO*, %) of the pomegranate red shell extract (1:100 dilution – triplicate analysis)

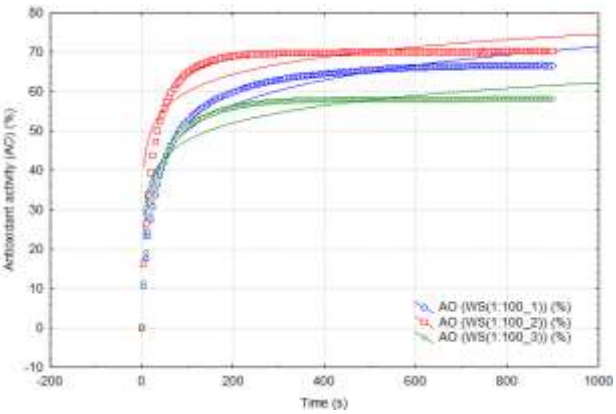


Figure 12. Variation of the antioxidant activity (*AO*, %) of the pomegranate white internal shell extract (1:100 dilution – triplicate analysis)

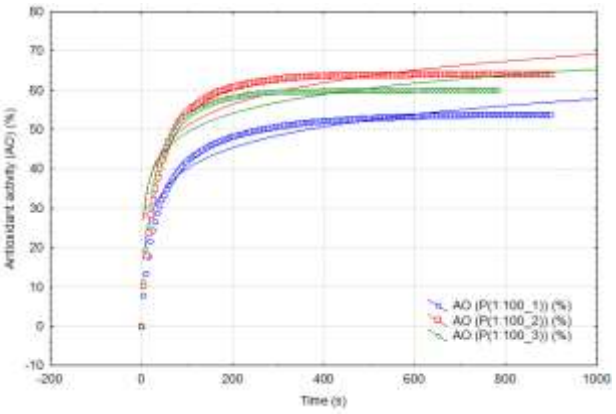


Figure 13. Variation of the antioxidant activity (*AO*, %) of the pomegranate pulp extract (1:100 dilution – triplicate analysis)

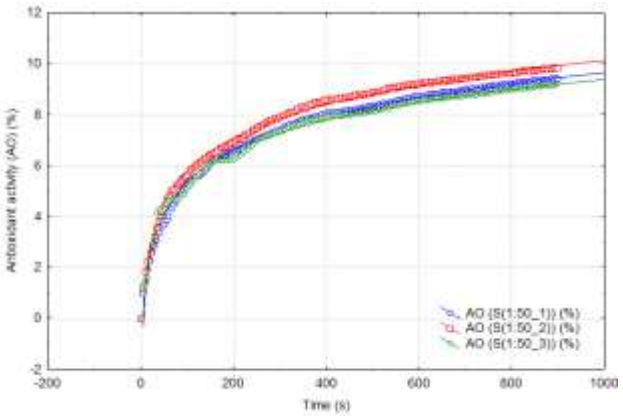
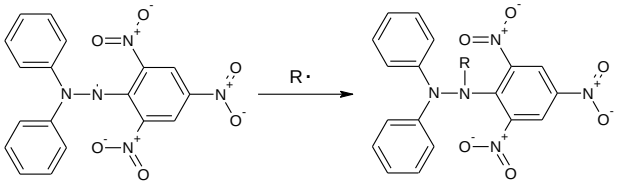


Figure 14. Variation of the antioxidant activity (*AO*, %) of the pomegranate seeds extract (1:50 dilution – triplicate analysis)

The kinetics of DPPH· reaction in the presence of pomegranate juices and alcoholic extracts were determined as the DPPH· mean reaction rates, taking into account the DPPH· standard curve for evaluation the actual and mean variation of the DPPH· concentration in time.

Pomegranate contains many organic compounds having antioxidant activity, almost all of them as enolic or polyphenolic derivatives (Figures 1-3). They belong to furanolactones (ascorbic acid, enolic derivative), hydroxybenzoic, hydroxycinnamic or cyclitol carboxylic acids (representative compounds are ellagic, caffeic, chlorogenic, or brevifolin), as well as flavonoids, anthocyanidinds and their glycosides (e.g., quercetin, rutin, naringenin, or delphinidin, cyanidin, and pelargonidin). There were many compounds having antioxidant activity, which were resulted by partial hydrolysis of tannins. It is the case of ellagitannin derivatives such as punicalin, punicaligin, punicafolin and punicacorteins, which are specific compounds from the secondary metabolism of *P. granatum* L.

The inhibition of a polymer chain radical derivative by antioxidant compounds such as polyphenols and enols can be modelled by DPPH·, a relatively stable organic radical (Scheme 1):



Scheme 1. Schematic reaction of DPPH· with a radical compound

The most important behavior of DPPH· in the presence of pomegranate juices and extracts (appropriately diluted) was observed up to 1½ minutes. This part was split into two time intervals of 0-30 s and 30-90 s, where the DPPH· concentration has pseudolinear variation. This variation divided by the corresponding time interval can directly provide the DPPH· mean reaction rates, suggesting the antioxidant behavior of the studied samples. However, some samples reveals some antioxidant activity even after the considered time interval of analysis (i.e., 15 minutes).

The DPPH· mean reaction rates are presented in Table 2 and Figures 15-23, for all three intervals of 0-30 s, 30-90 s, and 90-900 s. There are considerably higher for the first interval, especially for pomegranate red, white internal shell or pulp extracts, while these values had no significant differences for the second interval. Thus, The highest DPPH· reaction rates for the first interval for the above mentioned samples were in the range of 2.4-3 μM/s, while these reaction rates were only 0.33-0.85 μM/s for juice samples and even lower for seeds extract samples (0.17-0.2 μM/s) (Table 2).

Table 2. Mean DPPH· reaction rates in the presence of pomegranate juice and ethanolic extracts, for various DPPH· reaction time intervals

Nº	Code*	v_1** (μM/s)	v_2** (μM/s)	v_3** (μM/s)
1	*J(1:50_a)*	0.63 ± 0.15	0.30 ± 0.20	0.043 ± 0.002
2	*J(1:100_a)*	0.40 ± 0.00	0.15 ± 0.07	0.042 ± 0.023
3	*J(1:50_b)*	0.85 ± 0.49	0.25 ± 0.07	0.052 ± 0.005
4	*J(1:100_b)*	0.33 ± 0.21	0.13 ± 0.06	0.025 ± 0.01
5	*J(1:50_c)*	0.60 ± 0.14	0.20 ± 0.00	0.055 ± 0.023
6	*J(1:100_c)*	0.35 ± 0.07	0.15 ± 0.07	0.024 ± 0.003
7	*RS(1:50)*	2.97 ± 0.25	0.23 ± 0.12	0.005 ± 0.001
8	*RS(1:100)*	3.03 ± 0.06	0.47 ± 0.23	0.006 ± 0.001
9	*WS(1:50)*	2.83 ± 0.32	0.30 ± 0.00	0.003 ± 0.000
10	*WS(1:100)*	2.80 ± 0.52	0.60 ± 0.00	0.007 ± 0.004
11	*P(1:50)*	2.93 ± 0.15	0.37 ± 0.06	0.004 ± 0.001
12	*P(1:100)*	2.43 ± 0.38	0.60 ± 0.10	0.019 ± 0.006
13	*S(1:50)*	0.20 ± 0.00	0.07 ± 0.02	0.010 ± 0.001
14	*S(1:100)*	0.17 ± 0.06	0.05 ± 0.02	0.007 ± 0.002

* *J(1:50/100_a/b/c)* – pomegranate juice at 1:50 or 1:100 dilution (samples "*a*", "*b*" and "*c*"); *RS(1:50/100)* – pomegranate red shell ethanolic extract at 1:50 or 1:100 dilution; *WS(1:50/100)* – pomegranate white internal shell ethanolic extract at 1:50 or 1:100 dilution; *P(1:50/100)* – pomegranate pulp ethanolic extract at 1:50 or 1:100 dilution; *S(1:50/100)* – pomegranate seeds ethanolic extract at 1:50 or 1:100 dilution;

** Mean DPPH· reaction rates in the presence of pomegranate extracts on the following reaction time intervals: 0-30 s (v_1), 30-90 s (v_2) and 90-900 s (v_3)

There were no significant differences between 1:50 and 1:100 dilutions, especially for the most active samples (e.g., 2.97 and 3.03 μM/s, for the case of pomegranate red shell extract, diluted at 1:50 and 1:100, respectively, Table 2).

The second interval has DPPH· mean reaction rates of 0.05-0.60 μM/s), ten times higher for shell and pulp extracts in comparison with seeds extract samples and 2-5 times higher in comparison with the pomegranate juice samples. The DPPH· reaction rates in the presence of shell and pulp extracts were generally two times higher for less concentrated samples (1:100 dilutions) in comparison with 1:50 dilution samples, most probably due to the fast completing the DPPH· reaction in the presence of antioxidant compounds in higher concentrations (such as for 1:50 dilutions).

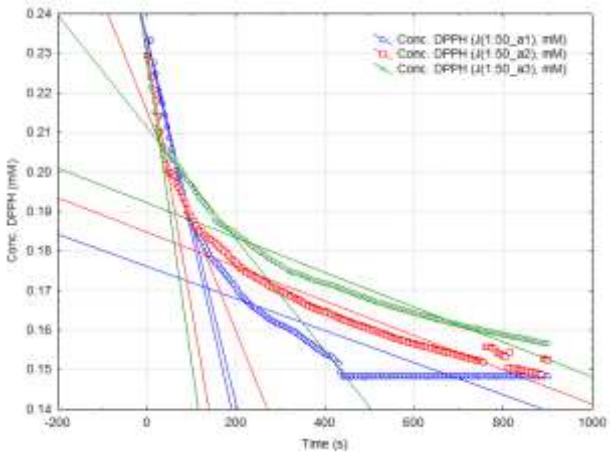


Figure 15. Variation of DPPH· concentration in the presence of pomegranate juice (sample "*a*" at 1:50 dilution – triplicate analysis)

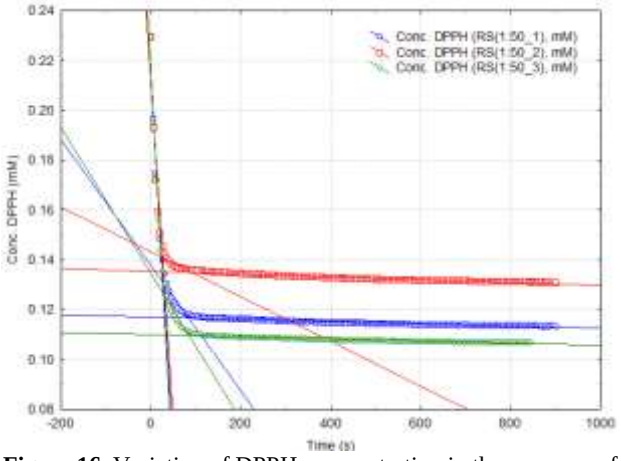


Figure 16. Variation of DPPH· concentration in the presence of pomegranate red shell extracts (1:50 dilution – triplicate analysis)

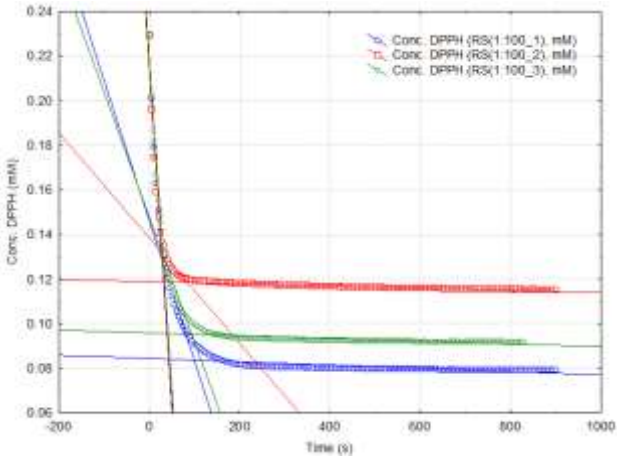


Figure 17. Variation of DPPH· concentration in the presence of pomegranate red shell extracts (1:100 dilution – triplicate analysis)

The corresponding values were 0.23-0.37 μM/s for the case of 1:50 dilutions and 0.47-0.60 μM/s for 1:100 dilutions (Table 2 and Figures 16-21).

However, the lowest values for the DPPH· mean reaction rates for this second interval was observed for seeds extract samples. No significant differences between values obtained for 1:50 and 1:100 dilutions were observed (0.07 μM/s and 0.05 μM/s, respectively; Table 2).

Interesting behavior was observed for the third interval. For the most concentrated extracts (shell and pulp extracts), as well as for less concentrated ones (seeds extracts), the DPPH· mean reaction rates had very low values of 0.003-0.019 μM/s, while these values were approximately ten times higher for juice cases (Table 2 and Figures 22,23).

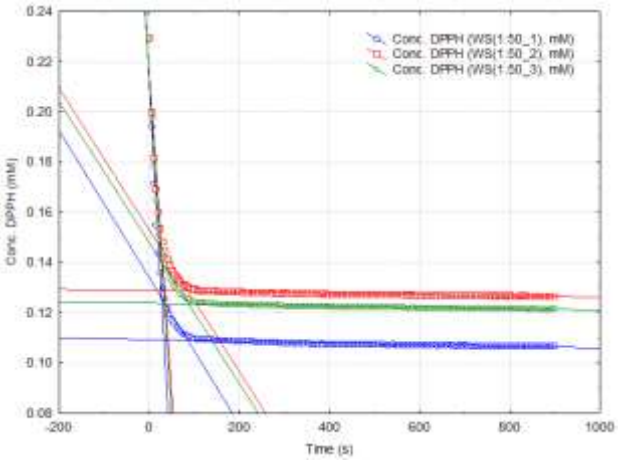


Figure 18. Variation of DPPH· concentration in the presence of pomegranate white internal shell extracts (1:50 dilution – triplicate analysis)

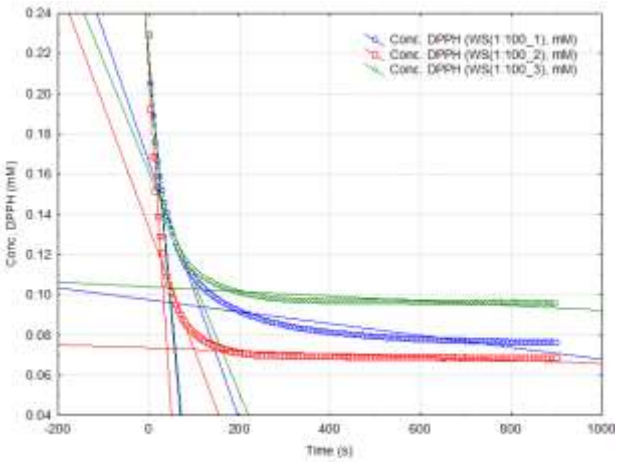


Figure 19. Variation of DPPH· concentration in the presence of pomegranate white internal shell extracts (1:100 dilution – triplicate analysis)

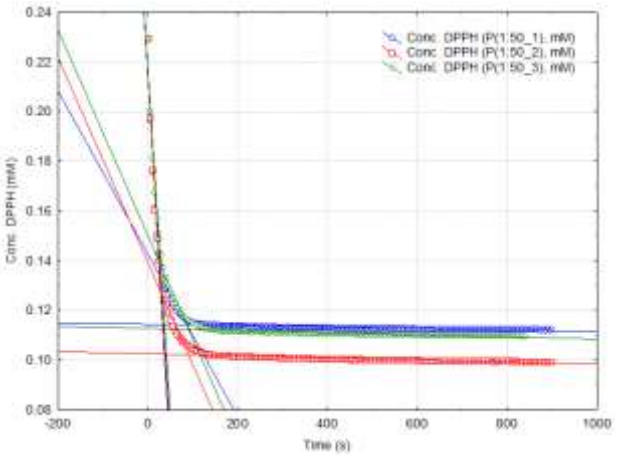


Figure 20. Variation of DPPH· concentration in the presence of pomegranate pulp extracts (1:50 dilution – triplicate analysis)

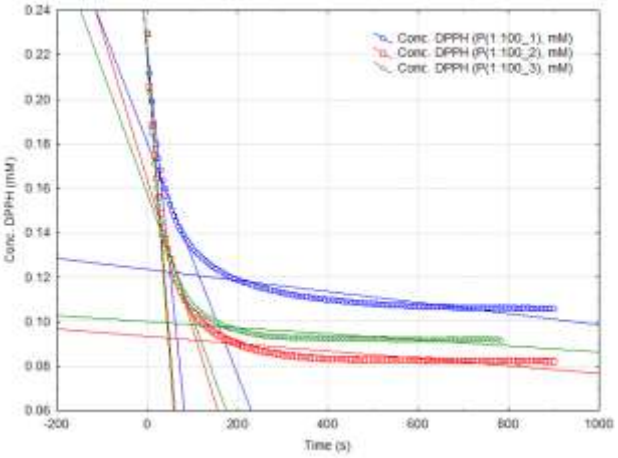


Figure 21. Variation of DPPH· concentration in the presence of pomegranate pulp extracts (1:100 dilution – triplicate analysis)

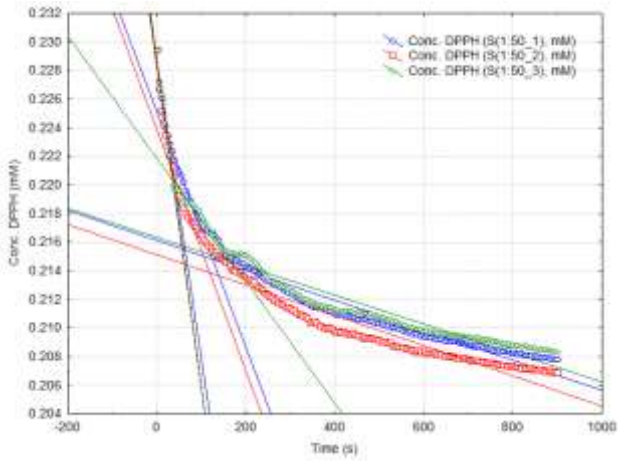


Figure 22. Variation of DPPH· concentration in the presence of pomegranate seeds extracts (1:50 dilution – triplicate analysis)

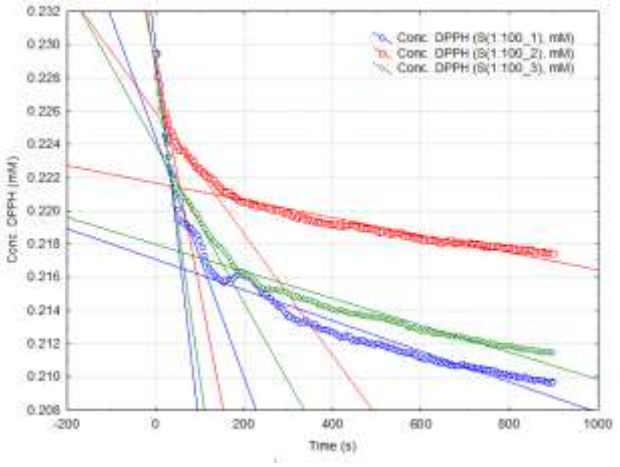


Figure 23. Variation of DPPH· concentration in the presence of pomegranate seeds extracts (1:100 dilution – triplicate analysis)

Thus, the mean reaction rates had values of 0.043-0.055 μM/s for the 1:50 dilutions and 0.024-0.042 μM/s for 1:100 dilutions (Table 2 and Figure 15).

4. Conclusions

In the present study, the kinetic evaluation of both pomegranate juice and hydroalcoholic extracts from various parts of pomegranate (*Punica granatum* L.) commercialized in the Banat region of Romania, in the presence of DPPH· radical have been performed. Actual antioxidant activity at various time of DPPH· interaction have also been determined.

The highest actual antioxidant activity was obtained for pomegranate red and white internal shell and pulp hydroalcoholic extracts, even at 1½ minutes of interaction, while the pomegranate juice had lower antioxidant activity, but this parameter had significant values even after fifteen minutes of monitoring.

The kinetic of the model radical compound reaction in the presence of antioxidant compounds from pomegranate juice and extracts (especially polyphenolic and enolic derivatives) reveals significant activity for hydroalcoholic extracts from red and white internal shell of pomegranate, which generally are considered as waste. Consequently, this study demonstrates the value of the pomegranate waste materials from the antioxidant activity point of view and suggest the recovery and re-utilization of such by-products in the food, pharmaceutical or cosmetic fields.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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