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Testing antiplatelet and antioxidant activity of the extract of seven varieties of *Allium cepa* L.

Abstract: Background: The extracts of *Allium cepa* are known for their medical use: antioxidant, anti-inflammatory, antimicrobial, fibrinolytic and antiplatelet properties. Our study aims to establish, using *in vitro* tests, the antiplatelet and antioxidant character, the link between them and the extract acidity, from seven varieties of *A. cepa*. Methodology: The qualitative and quantitative presence of polyphenols and anthocyanins in the extracts was determined using UV-Vis and HPLC. Quantitative determination of the thiosulfinates compounds was calculated using their reaction with 4-mercaptopyridine. Antioxidant character was determined using 3 methods (FC, DPPH and TEAC), and antiplatelet effect was measured by *in vitro* tests on platelet rich plasma obtained from human blood. Principal Findings/Results: The white variety of *A. cepa* has the most alkaline pH, the largest amount of thiosulfinate compounds and the most powerful antiplatelet effect, but a very small amount of flavonoids and an antioxidant effect almost nonexistent, in contrast with red variety of *A. cepa* which is the opposite. Conclusions/Significance*:* The white variety of *A. cepa* had very high anitiplatelet activity suggesting the potential use of *A. cepa* extract in treating cardiovascular diseases.

Keywords: antiplatelet activity; antioxidant activity; Allium cepa; pH; thiosulfinates

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1 Introduction

Studies on and the medical use of *Allium cepa* L. phytoconstituents, mainly thiosulfinate and flavonoid compounds, have gained significant attention in the past few years, since some of these show promising antioxidant, antidiabetic, anti-inflammatory, anticancer, antimicrobial, antihyperlipidemic, fibrinolytic, antiplatelet, immunomodulatory and anti-ischemic activity [1-3].

During sectioning of the bulbs of *A. cepa*, S-alk(en)yl-L-cysteine sulfoxides are metabolized by the action of alliinase generating thiosulfinate compounds [4]. These thiosulphonates are not only responsible for the specific flavor of onion, but also play a significant part towards antiplatelet activity [4, 5]. Despite the fact that the biosynthesis pathway of the organosulfur compounds from *A. cepa* has been well studied, the mechanism through which these compounds inhibit platelet aggregation is not yet completely understood [4-6].

In this context, the attention of some studies has been directed towards the standardization of extraction conditions before testing antiplatelet activity [3]. During a pilot study, researchers have noted that the antiplatelet activity of the *A. cepa* extract increases if this is stored at room temperature for a period of time [7]. On the contrary, the freshly prepared extract that is left at room temperature for less than 2 minutes leads to a pro-aggregant response on platelets in human blood plasma. This observation suggests the presence of pro-aggreganting compounds in the fresh extract of *A. cepa*, prior to the formation of thiosulfinate compounds [7]. Fresh tissue extract contains intact and broken mitochondria, which indicates the presence of ADP (and ATP) [7], a potent activator of platelets [8].

The *in vitro* effects of *A. cepa* extract have been examined by monitoring the metabolism of arachidonic acid (AA) from the platelets. *A. cepa* extract seems to reduce the formation of thromboxane A_2 (TxA_2) and of the products resulting via lipoxygenase pathway, from exogenous AA; it does not inhibit AA incorporation into platelet phospholipids. Thus, it has been shown that the extract inhibits the formation of AA metabolites, acting one step after its release. At extract concentrations that suppress the formation of AA (inducer of platelet aggregation), only a partial inhibition of aggregation in the presence of ADP and epinephrine has been observed. These results suggest that the inhibition of platelet aggregation in the presence of the *A. cepa* extract is mediated by its effects on the synthesis of TxA2 [5]. It is also known that platelet response induced by the *A. cepa* extract is time and temperature dependent, with the mention that the maximum effect is observed when the extract is preserved over 30 minutes at a temperature of 25°C [4]. *Ex vivo* platelet aggregation is classically measured using aggregation tests with plasma or whole blood [9].

Given that most of these studies are focused on examining the mechanism of platelet aggregation or the relative strength of the extracts [10], as a novelty in the field, this study will evaluate the relation between the composition of *A. cepa* extract (the amount of flavonoids/ thiosulfinates), its antioxidant character and its antiplatelet activity. Although the antioxidant character of *A. cepa* extracts is well known, there are no studies that link the antioxidant and antiplatelet activities with parameters describing the quality of the extract.

2 Experimental Procedures

2.1 Preparation of *A. cepa* extracts

Seven varieties of onions were evaluated in this study, six from different regions of Romania and one from Hungary, grown on different soils. Details about these varieties are listed in Table 1. Bulbs of *A. cepa,* cleaned and portioned, were subjected to triturating and squeezing processes using an electric juicer (**Electrolux, model EBR5050**). The extract was left at room temperature for 30 minutes, time required to complete the conversion of S-alk(en) yl L-cysteine sulfoxides into thiosulfinate compounds in the presence of alliinase, targeted for their antiplatelet effect. The extract thus obtained was subjected to centrifugation (**Beckman centrifuge, model J2-J1**) for removal of cell wall debris. After centrifugation for 10 min at 13000 rpm, the supernatant was recovered, which is considered the aqueous extract of *A. cepa* (fresh juice). The aqueous extract was stored at -20 °C; it was thawed on ice immediately before use. Immediately after obtaining the extract, the pH for each sample was determined (**Hanna Instruments, pH 212 model**). The solid residues that remained after obtaining the aqueous extract were re-suspended in a 90% (v/v) ethanol solution, equivalent to the quantity of the resulting aqueous extract. The ethanol extract thus obtained was left for 24 hours at a temperature of 5 °C, and then centrifuged for 10 minutes at 13000 rpm; the recovered supernatant was considered the residual ethanol extract of *A. cepa*. To determine the dry residue and water content of the aqueous extract, a portion of the freshly obtained juice was subjected to freezing (-20 °C) and then lyophilization (**Lyophilizer – Model Alpha 1-4LDPLUS**).

2.2 Determination of the antioxidant capacity of *A. cepa* extracts

The antioxidant capacity of *A. cepa* extracts was evaluated using three different methods, which are the following. 1. Folin-Ciocalteau assay. Using the Folin–Ciocalteu reagent prepared as described in other papers [11]. All measurements were performed in triplicate. For the gallic acid standards, a calibration curve (Figure S1, Supplementary data) was made ($R > 0.999$, $p < 0.001$) and the total electron-rich component level (mostly known as total phenolic content) for each sample was determined in terms of gallic acid equivalents (μg GAE / mL sample). 2. DPPH bleaching assay. For each sample, six different aliquots of suitable volumes depending on the samples were added to a proper volume of DPPH ethanol solution (100 μM), so that the final volume was 1000 μL in the quartz cuvette and the bleaching of DPPH was kinetically monitored for 25 min at 517 nm using a UV-vis spectrophotometer (**Varian, Cary 50**) equipped with a multi-cell holder. Typical decay curves were obtained for every sample (Figure S2, Supplementary data). The 517 nm absorbance was corrected for the dilution effect. The percentage of DPPH remained unbleached after the reaction time was calculated for all tested volume samples and using a plot of these values vs. extract concentration, a curve was generated which was fitted by a first order exponential decay function allowing the calculation of EC_{50} (efficiency concentration as defined by Sanchez-Moreno, Larrauri and Saura-Calixto [12], Figure S3, Supplementary data). Since the samples are both fresh juice and their residual ethanol extract, the final values of EC_{50} are given in volume units. The smaller the EC_{50} value the greater the antioxidant capacity. 3. TEAC (Trolox equivalent antioxidant capacity) assay, using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical obtained by enzymatic action (peroxidase). The percentage of the consumed ABTS radical was calculated based on absorbance and a blank (no sample added) for each sample in triplicates. At the same time, similar tests were performed by using trolox standards at 3, 6, 9, 12, 15 µM. Based on these standards, a calibration curve (Figure S4, Supplementary data) was constructed (R > 0.999) and the percentage of the consumed ABTS radical for each sample was converted to trolox equivalents (TE) and expressed in µg TE/ mL sample.

2.3 Determination of the antiplatelet effect of *A. cepa* extracts

The antiplatelet effect was determined using platelet-rich plasma (PRP). The principle of the method is based on the decrease in plasma OD at the wavelength of 600 nm (**Varian, Cary 50**), caused by the addition of a platelet aggregation agonist with the formation of platelet thrombi. Blood was collected from healthy human subjects, who had no long-term medication or antiplatelet drugs within the last 7 days. Patients agreed to participate in the study by signing an informed consent. Blood was collected in a sodium-citrate solution in a ratio of 9:1 (v:v), kept at room temperature and used within 2 hours. To obtain platelet-rich plasma (PRP), the blood was centrifuged at 185 g for 10 minutes and to obtain platelet-poor plasma (PPP), the blood was centrifuged for 15 minutes at 1500 g (**Centurion Scientific, K3 Series swing-rotor centrifuge**). Using a plastic pipette, PRP and PPP were separated and transferred to polypropylene tubes. A dilution of plasma with a PBS solution (2 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, with the pH adjusted to 7.4) was prepared, in a ratio of 50:50 (v:v). Initially, the spectrophotometer was calibrated using PPP, which is considered 100% transmittance, and PRP 0% transmittance.

The optimal conditions were established after numerous preliminary tests. The temperature was established at 37 °C, by stirring continuously the samples during the entire experiment using a magnetic stirrer set at 500 rotations/minute. There were two control samples and two test samples for each variety of *A. cepa*, by taking into account the average of the measurements. For control samples, after 5 minutes of pre-incubation at 37 °C, the optical density (OD) reading was started; after one minute of OD monitoring, the platelet agonist was added following the OD monitoring for 5 min. In this case, the platelet agonist used was AA (**Sigma-Aldrich, from porcine liver, USA**), for the reasons mentioned above. For control samples, it was added to each sample (2 mL of PRP: PBS solution) a volume from a stock solution of AA in DMSO, with a final concentration of 0.683 mM. For the extracts, a predetermined amount of aqueous *A. cepa* extract was added at 30 seconds, and AA solution 0.683 mM, at 1 minute after the start of the absorbance reading; the absorbance reading continued up to 5 min. Two different methods were employed to calculate the antiplatelet effect, as described below. Method 1 (abbreviated M_1) measures the percentage of inhibition of platelet aggregation using the formula:

$$M_1 = 100 - (X/Y) \times 100,$$

where X is OD_{600nm}, at the end of the reaction (after 5 minutes) for the test sample and Y represents OD_{600nm}, at the end of the reaction (after 5 minutes) for the control sample, while method 2 (abbreviated M_2) uses the slope of the kinetic curves at 600 nm, based on the formula:

$$M_2 = 100 - (s_1/s_c) \times 100,$$

where S_1 is the slope of the linear part of the curve for the test sample and S_c is the slope of the curve for the control sample. The calculation of the two parameters (M_1, M_2) based on these two methods is illustrated in Figure 1a.

2.4 Determination of the total thiosulfinates compounds content in *A. cepa* extracts

Thiosulfinates are more stable in aqueous solution, than in ethanolic one [13], thus only the onion juices were assessed for the thiosulfinate content. Recently, an assay for the determination of total thiosulfinate content (TTC) was published [14] and is used in the current study. Thus, to dertermine the thiosulfinate compounds from aqueous extracts, a reaction with 4-mercapto-pyridine (4-MP) (**Sigma Aldrich, Steinheim, Germany**) was employed. An ethanolic stock solution 36.1 mM 4-MP was prepared, as well as a buffer solution of 50 mM sodium phosphate and 2mM EDTA, pH 7.2. The stock solution of 4-MP was diluted fifty times in the buffer mentioned above (final concentration 772 µM). The *Allium* extract was incubated at room temperature in the buffer solution of 4-MP mixed in a ratio of 15:85 (v:v). Two replicates for each variety of *A. cepa* and a control sample were run. The OD at a wavelength of 324 nm was measured every 5 minutes, for one hour using a spectrophotometer (**Varian, Cary 50**). The TTC was evaluated by the kinetic constant k of the kinetic curves at 324 nm, after fitting the experimental data with a first order exponential decay function.

2.5 HPLC analysis of *A. cepa* extracts

The experiments were carried out using an **Agilent 1200 HPLC series system** consisting of a quaternary gradient pump, temperature-controlled autosampler with a 100 µl injection loop and a diode array detector. For the separation, a reversed-phase Agilent Zorbax ODS column (5 µm, 4.6 x 250 mm) was used. All chromatographic operations were carried out at ambient temperature. Flow rate and injection volume were 1.0 ml /min and 40 µl. Detection of all compounds was performed at 254nm, 290nm, 340nm, 380nm and 520nm. The mobile phases were methanol (phase A) and 0.1% formic acid solution (v/v) (phase B). The elution method was optimized during numerous preliminary experiments using nine standards (hydroxybenzoic acid, vanilic acid, caffeic acid, coumaric acid, ferulic acid, rutin, quercetin, luteolin, kaempferol), which are some of the possible phytoconstituents in onion extracts. All solvents were HPLC-grade and chemicals were analytical grade. All solvents were filtered through 0.5 µm filters and degassed in an ultrasonic bath. The elution began with an isocratic step (90 % B) for the first 10 min, followed by multistep gradients as follows: 10 – 70 min, the phase B linearly decreased from 90 % to 45 % and during 70 – 75 min interval from 45 % to 20 % ending during 75 – 85 min from 20 % back to 90 % B. Some of the previously mentioned phytoconstituents were identified in the onion extracts based on retention time and spectral matches (UV Spectra with those of the reference standards) and the content of quercetin and rutin from both fresh juice and residual ethanolic solution (the two main important flavonoids in onions) were determined using external standard method. The obtained dates were processed with Chemstation software (**Agilent, USA**).

2.6 Statistical analysis

All the measurements were taken in multiple replicates and the standard deviation and standard error of the mean were calculated for evaluating the precision of the measurement. Statistical analysis was performed using *Statistica 7.0* for *Windows* (Stat-Soft, Inc., USA). Box and Whisker plot and Pearson correlation were used to examine the strength of associations between the results. The experimental data were evaluated using the classical ANOVA one-way analysis of variance. Multivariate data analysis was performed on the entire antioxidant and antiplatelet parameters determined in this study using PCA (Principal Component Analysis) incorporated in *Statistica* software. The main purpose of PCA is to conveniently represent the location of the objects (samples) in a reduced coordinate system where, instead of m-axes (corresponding to m variables), only p ($p < m$) are used to describe the data set with the maximum possible information (in well models, two or three components contain almost all information from the primary matrix) [15].

3 Results and discussion

In the process of obtaining the *A. cepa* juice, the seven onion varieties have a different juice content, between 44 and 65% (mL/g material) (see Table 1). The juice content apparently does not depend on the type of onion but rather on variety. While the white variety has the lowest juice content, 43.6%, and red onions have a mean value of 61% juice content, yellow ones have values between 45-65%, depending on the variety, with an average of 58% (Table 1). The dried fraction of the juice (lyophilized fraction) was determined for each variety (Table 1) and as expected, it was highly negatively correlated with the juice content (Figure S5, Supplementary data), meaning that the higher the juice content, the smaller the dried material fraction was (except sample 4). Both of these parameters are very important for food industry and for possible pharmaceutical preparations from onion juice. However, these parameters are not correlated at all with any other antioxidant or antiplatelet parameter (*vide infra*). Interestingly, it was observed that the pH of the fresh juices depended on the variety and type of onion, the red varieties of *A. cepa* (samples 6 and 7) had a more acidic pH (5.07 ± 0.00) than the yellow ones (5.40 ± 0.10) and the white variety (sample 3), which was the most alkaline (5.54) (see Table 1). These differences in pH may indicate important differences in chemical composition (thus, differences in quality) and probably differences in stability.

3.1 The antiplatelet effect of A. cepa extracts

The two methods described in the *Materials and methods* section (M1. M2) are illustrated in Figure 1a and are useful for determining the antiplatelet activity of the onion juices. Method 1 carries quantitative information

Table 1: Onion (*A. cepa*) varieties used in the study and their juice content; lyophilized fraction, pH, total thiosulfinate content (TTC), antiplatelet effects.

Sample No.	Variety	Color	Sample weight (g)	Sample juice volum (mL)	Juice content (%)	Df[a] (%)	pH	M_1[b] (%)	M_2[c] (%)	TTC[d] (k_1 - min^{-1})
1	Buzău Golden Onion (Aurie de Buzău, RO)	Yellow	378.3	170	44.9	14.8	5.47	88.05	83.95	0.040
2	Buzău Onion (De Buzău, RO)	Yellow	413.3	240	58.1	13.6	5.34	92.63	88.41	0.040
3	White Hungarian Onion (Albă de Ungaria, HU)	White	286.6	125	43.6	14.9	5.54	95.23	98.57	0.115
4	Braşov Onion (De Braşov, RO)	Yellow	480.9	300	64.2	16.0	5.50	94.14	96.06	0.025
5	Yellow Constanţa Onion (Galbenă de Constanţa, (RO)	Yellow	536.4	350	65.2	12.7	5.30	53.73	83.47	0.005
6	Ruby Onion (Rubiniu, RO)	Red	402.3	250	62.1	13.1	5.07	10.69	89.57	0.036
7	Arieş Red Onion (Roşie de Arieş, RO)	Red	288.1	170	59.0	13.3	5.07	22.51	84.38	0.043

[a]Df – Lyophilized fraction (%, dried material after lyophilization/volume of onion juice), [b]M_1 – Platelet aggregation inhibition by method 1, [c]M_2 – Platelet aggregation inhibition by method 2, [d]TTC –reaction rate constant between 4-MP and onion juice, a measure of thiosulfinate

regarding antiplatelet activity, while method 2 mainly brings qualitative information. A clear inhibition of platelet formation triggered by AA was observed for each sample. Preliminary tests using sample 1 indicated that this inhibitory effect was dose dependent (Figure 1b).

Despite the fact that even a small volume of 5 µL caused a significant inhibition of platelet aggregation in the case of sample 1, a volume of 20 µL was further used as an optimal volume since other samples, even at this volume have a small but significant effect. The platelet aggregation curves in the presence of onion juices together with the antiplatelet activity results (M1) are depicted in Figure 1c. It may be observed that both the type and the variety of the onion influence this activity. The red varieties of *A. cepa* (samples 6 and 7) and one yellow variety (sample 5) have a much lower antiplatelet activity than the other yellow ones and the white variety, which has the strongest antiplatelet activity (Figure 1c,d). It is very interesting that this activity is strongly correlated to the pH of the sample ($R = 0.946$, $p < 0.000$). While M1 values are well differentiated between the samples, M2 values are much more similar, showing that the quality of the process (or even mechanism) is very similar for all samples.

Thiosulfinates are mainly responsible for the antiplatelet activity of the onion extracts [4, 5]. For this purpose, in this study a recent method designed for the evaluation of total thiosulfinate content (TTC) was used. Using 4-MP as a chromogenic thiol (324 nm), which is a stable and inert compound and does not interact with oxidized glutathione and cysteine, the thiosulfinate compounds from the *Aliaceae* extracts can be quantified [16]. The principle of the method is based on the reaction of 4-MP with the thiosulfinate compounds (activated disulfide bond from the thiosulfinate compounds-S (O)-S-), leading to 4-MP consumption during the chemical reaction, with the formation of mixed disulfide, 4-allyl-mercapto-pyridine. The consumption rate of 4-MP in the reaction was monitored spectrophotometrically, following the model of other authors [16,17], which showed that there is a linear relation between the amount of thiosulfinate compounds and their reaction rate. The reaction rate constant (k) was calculated, at 24°C and at pH = 7.2 for each sample, from a typical first order exponential decay curve. The kinetic constant k is used as a parameter to describe the TTC in the samples. The highest concentration of thiosulfinate compounds was identified in sample 3, the white variety of *A. cepa* (Table 1). No significant correlation was observed between TTC and M1 values and there was a poor correlation with M2 values. This fact may indicate that it is not the TTC that is important for antiplatelet activity, but rather a distinct thiosulfinate class is mainly responsible for the activity. Further investigations are needed in this direction.

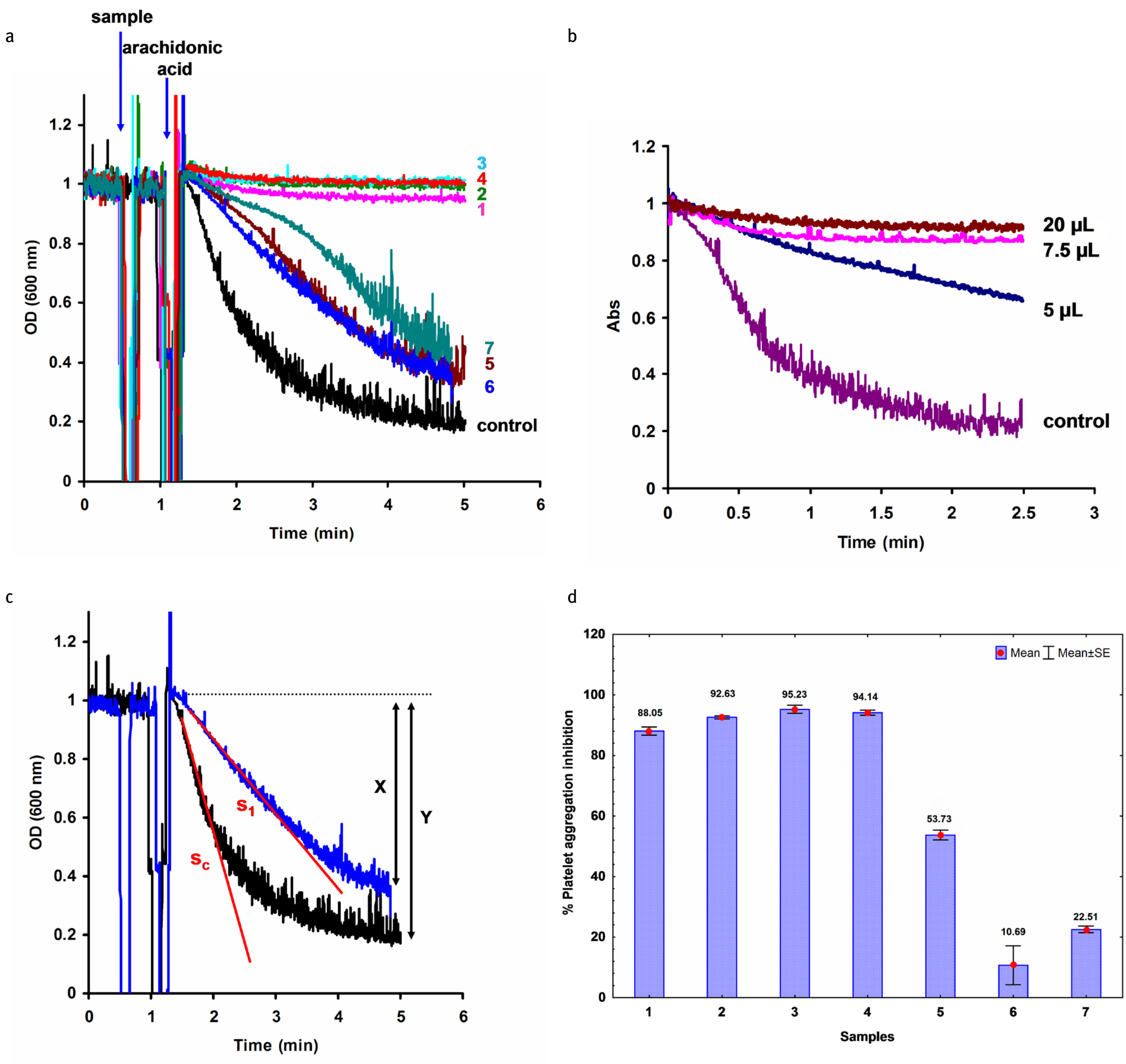

Figure 1. **(a)** Illustration of the two methods (M_1 and M_2) employed to measure the antiplatelet effect of *A. cepa* extracts; the black curve is the control, while the blue curve is the real sample (sample 6); **(b)** Profiles of platelet aggregation curves induced by AA, in the presence *A. cepa* extracts at different extract volumes (5 mL, 7.5 mL, and 20 µL *A. cepa* extract (sample 1)); **(c)** Profiles of platelet aggregation curves induced by AA, in the presence of various varieties of *A. cepa* extracts (20 ml sample for 2 mL PRP:PBS (1/1, v/v), AA 0.683mM, final concentration); **(d)** Results of inhibition of platelet aggregation induced by AA using M_1, in the presence of onion juices of various *A. cepa* varieties (M2 parameters are listed in Table 1) (ANOVA test, $p < 0.001$).

3.2 The antioxidant capacity of A. cepa extracts

The three methods employed for the evaluation of the antioxidant activity of the extracts, DPPH bleaching assay, Folin-Ciocalteu reagent based assay (abbreviated GAE) and TEAC assay, are among the most used *in vitro* methods for this purpose. The DPPH bleaching assay implies an assessment run in ethanol solution and the TEAC and GAE assays are based on aqueous solutions. All of these methods are based on the electron transfer mechanism [18]. The antioxidant capacities of the fresh onion juices, using all these methods, as well as for the residual ethanol extracts, are plotted in Figure 2. GAE and TEAC equivalents as well as the antioxidant efficiencies ($1/EC_{50}$), typical for natural extracts, have the same pattern for all studied samples. The red onion varieties have the most antioxidant activity, followed by the yellow ones, the white variety having the lowest antioxidant activity. It is very interesting that this order is opposite to antiplatelet activity. It is useful to mention that the residual ethanol extracts also have a potent antioxidant activity (just slightly less that the fresh juices), suggesting that a lot of important antioxidant compounds are still present in the residuals. This may be explained by the low solubility of flavonoids and phenolic compounds in the fresh aqueous juices. Despite the fact that an ethanol extract of the entire plant material would lead to an extract richer in antioxidant activity, the stability of thiosulfinates would be greatly affected and the antiplatelet activity would not be maximally retained. Thus, depending on the interests, one may decide the type of extraction necessary.

The main phytoconstituents known to be responsible for the antioxidant effect in onions are flavonoids (mainly quercetin and rutin) and some phenolic acids. The HPLC method employed in our study was useful for the quantification of rutin and quercetin. The results are from these experiments are tabulated in Table S1 (Supplementary data). Flavonoids in sample 3 were below detectable limits (Figure S6 and Table S1), in good agreement with antioxidant capacity results. The UV-vis spectra of the onion juices (Figure S7, Supplementary data) revealed the presence of flavonoids (about 360 centered band) and the maximum spectral absorbance were in very good agreement with antioxidant capacity data (R = 0.831), suggesting that the antioxidant activity was mainly attributed to these flavonoids. The white onion variety presents no flavonoids as determined also by HPLC.

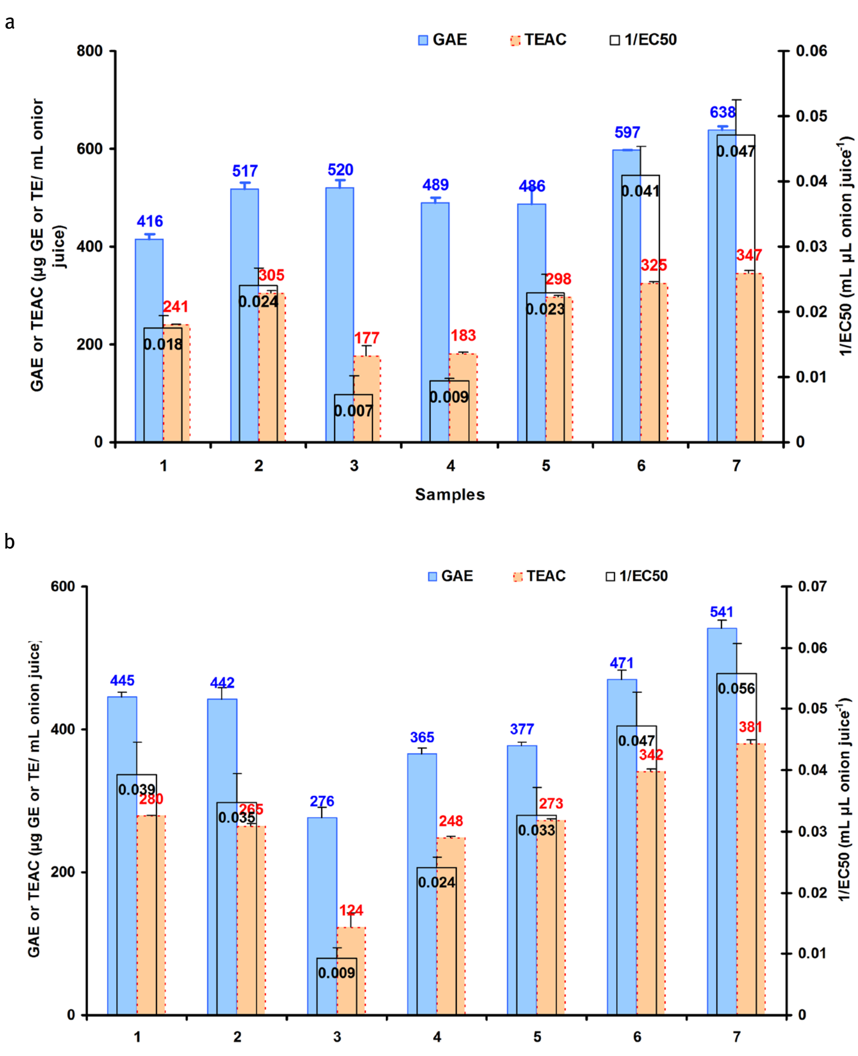

Figure 2. (a) Antioxidant capacities of *A. cepa* juices; **(b)** Antioxidant capacities of residual ethanolic extracts - using the GAE (Folin-Ciocalteu based assay), TEAC and DPPH bleaching assays (ANOVA test, $p < 0.001$).

3.3 Correlations between the antiplatelet effect, antioxidant capacity and other parameters describing the quality of A. cepa extracts

The correlations between the parameters describing antiplatelet activity (M1, M2) and those describing antioxidant capacity (TEAC, DPPH ($1/EC_{50}$) and GAE), as well as pH are plotted in Figure S8 (Supplementary data). For a better evaluation of this correlation, a PCA approach was employed. After applying PCA on a matrix containing all parameters, new variables (called principal components) were obtained and they were represented by a linear combination of the primary variables (in our case the antiplatelet, antioxidant parameters and pHs of the samples). The loadings and scores are the main results of the PCA model where the loading indicate the relative importance of the corresponding original parameters in each principal component and the scores represent the new coordinates corresponding to the principal component for every sample [15]. In our case, the first three principal components explain 96.79% of total variance (Figure S9, Supplementary data).

The obtained scores and loading plots are very useful as a display tool for examining the relationships between the original parameters, looking for trends, and sorting out outliers [15,19-22]. The loading plots for the determined antiplatelet, antioxidant parameters and pHs are presented in Figure 3 and the correlation coefficients are given in Table 2. In Figure 3a, it may be observed that the antioxidant parameters are in the left part of the correlation circle, while the antiplatelet activity and pH are in the right one, indicating a negative correlation between them. It may also be noted that M1, pH, TEAC and $1/EC_{50}$ parameters (parameters along the first principal component) are well correlated. The TCC is much closer to M2 than to M1 since the antiplatelet activity is differently calculated between the two methods M1 and M2, because one calculates the maximum inhibition of the reaction, and the other one calculates the speed of the reaction of platelet aggregation inhibition. This result of negative correlation between the antiplatelet activity and antioxidant capacity of the onion juices is very important for the food quality involving onion processing. Furthermore, the high correlation between pH and these two activities needs further investigation. This difference may be due to the differences in the chemical composition of some phytoconstituents. An illustration of the dependence between M1, TEAC and pH can be found in Figure 3c. It is obvious that onions with a pH higher than 5.5 have poor antioxidant activity but are rich antiplatelet products (mainly the white onion and sample 4), while those with a pH lower than 5 are food sources of antioxidants, but have less antiplatelet activity (red *A. cepa* variety).

The score plot of the first three principal components (Figure 3b) indicates a clear grouping of the *A. cepa*, according to their type and variety based on pH, antiplatelet and antioxidant parameters. According to Figure 3b, sample 4 (Brașov *A. cepa* variety, with the richest dried content) appears to be distinct among all the yellow *A. cepa* varieties, being close to the white variety in terms of antiplatelet and pH values.

Table 2: Correlation matrix (containing the correlation coefficients) between the antioxidant parameters described in the current paper. Values over 0.800 are presented in bold numbers.

	TEAC	1/EC50	GAE	pH	M1	M2	TTC
TEAC	1.000	**0.955**	0.595	**-0.914**	-0.778	-0.750	-0.468
1/EC50		1.000	0.404	**-0.844**	-0.717	**-0.844**	-0.630
GAE			1.000	**-0.803**	-0.756	-0.005	0.113
pH				1.000	**0.946**	0.525	0.358
M1					1.000	0.446	0.302
M2						1.000	0.633
TTC							1.000

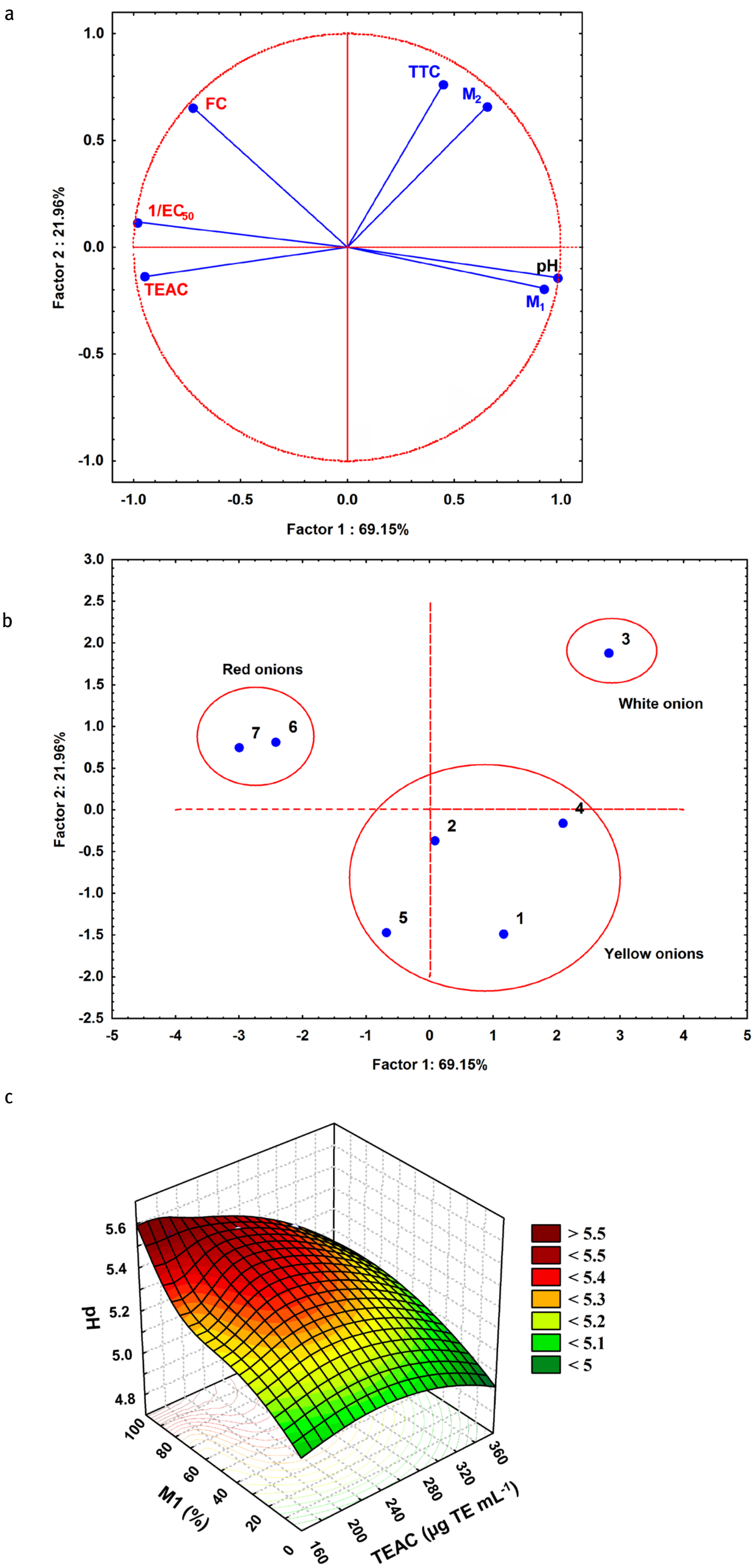

Figure 3: (a) Correlation circle (loading plot) using the first two principal components of the PCA model obtained after applying PCA; **(b)** Score plot using the first three principal components of the seven onion varieties; **(c)** Dependence between antiplatelet activity (M1), antioxidant activity (TEAC) and pH values for the studied *A. cepa* extracts.

4 Conclusions

The current study discussed the antioxidant and antiplatelet properties of several *A. cepa* varieties. *A novelty* is the significant correlation observed between the acidity, the antioxidant capacity and the antiplatelet effect of the *A. cepa* extracts. It was observed that the red varieties of *A. cepa* had the strongest antioxidant activity using all three methods (FC, TEAC, DPPH), and the lowest platelet inhibition effect compared to the other varieties. The white variety of *A. cepa* extract had the most powerful antiplatelet effect and an almost non-existent antioxidant capacity. Principal component analysis (PCA) reveals informative classifications based on the antioxidant and antiplatelet activities and their correlations. Regarding the antiplatelet effect, which is very strong for the white variety of *A. cepa*, this study could be the starting point in obtaining and testing an *A. cepa* extract, which can be used in cardiovascular diseases.

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