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Relationships Between Bioactive Compound Content and the Antiplatelet and Antioxidant Activities of Six *Allium* Vegetable Species

Hebe Vanesa Beretta[1], *Florencia Bannoud*[1], *Marina Insani*[2], *Federico Berli*[1,3], *Pablo Hirschegger*[3], *Claudio Rómulo Galmarini*[1,3,4] *and Pablo Federico Cavagnaro*[1,3,4]*

[1]National Council of Scientific and Technical Research (CONICET), Av. Rivadavia 1917, C1033AAJ Buenos Aires, Argentina

[2]National Institute of Agricultural Technology (INTA), Nicolas Repetto y de los Reseros s/n, Hurlingham, 1686 Buenos Aires, Argentina

[3]National University of Cuyo, Faculty of Agricultural Sciences, Almirante Brown 500, Luján de Cuyo, 5505 Mendoza, Argentina

[4]National Institute of Agricultural Technology (INTA) – E.E.A. La Consulta, La Consulta CC8, San Carlos, 5567 Mendoza, Argentina

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Summary

Allium sp. vegetables are widely consumed for their characteristic flavour. Additionally, their consumption may provide protection against cardiovascular disease due to their antiplatelet and antioxidant activities. Although antiplatelet and antioxidant activities in *Allium* sp. are generally recognised, comparative studies of antiplatelet and antioxidant potency among the main *Allium* vegetable species are lacking. Also, the relationship between organosulfur and phenolic compounds and these biological activities has not been well established. In this study, the *in vitro* antiplatelet and antioxidant activities of the most widely consumed *Allium* species are characterised and compared. The species total organosulfur and phenolic content, and the HPLC profiles of 11 phenolic compounds were characterised and used to investigate the relationship between these compounds and antiplatelet and antioxidant activities. Furthermore, antiplatelet activities in chives and shallot have been characterised for the first time. Our results revealed that the strongest antiplatelet agents were garlic and shallot, whereas chives had the highest antioxidant activity. Leek and bunching onion had the weakest both biological activities. Significantly positive correlations were found between the *in vitro* antiplatelet activity and total organosulfur (R=0.74) and phenolic (TP) content (R=0.73), as well as between the antioxidant activity and TP (R=0.91) and total organosulfur content (R=0.67). Six individual phenolic compounds were associated with the antioxidant activity, with catechin, epigallocatechin and epicatechin gallate having the strongest correlation values (R>0.80). Overall, our results suggest that both organosulfur and phenolic compounds contribute similarly to *Allium* antiplatelet activity, whereas phenolics, as a whole, are largely responsible for antioxidant activity, with broad variation observed among the contributions of individual phenolic compounds.

Key words: *Allium* sp., garlic, onion, antiplatelet activity, antioxidant activity, phenolic compounds

*Corresponding author: Phone: +54 261 4605 737; E-mail: pablocavagnaro@hotmail.com

ORCiD IDs: 0000-0001-9245-5640 (Beretta), 0000-0002-4813-9429 (Bannoud), 0000-0003-1654-0549 (Insani), 0000-0003-1235-5901 (Berli), 0000-0001-5875-9640 (Hirschegger), 0000-0002-7958-0110 (Galmarini), 0000-0001-5838-0876 (Cavagnaro)

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, with higher prevalence in developed countries. Foods with antiplatelet and antioxidant compounds can reduce the risk of CVD by maintaining haemostasis and aiding in the prevention of thrombosis and oxidative stress (*1*). Among *Allium* sp. functional properties that may confer protection against CVD, antiplatelet activity has been extensively studied in garlic (*2–5*) and onion (*5–8*), and in selected organosulfur compounds from these species (*9*). Antiplatelet agents have also been found in leek (*10*) and bunching onion (*11*). These studies used very different experimental conditions (*e.g.* different sources of platelets, agonists and aggregometer apparatus; and different sources of the antiplatelet agents, such as fresh or cooked plant materials, or isolated compounds, *etc*.) and they only analysed antiplatelet activity of a single *Allium* species, making their results not directly comparable. Thus, comparative analysis of the antiplatelet efficacy of the main *Allium* vegetables, using standardised experimental procedures, would be of great interest. Also, antiplatelet activity has not been investigated in chives and shallot. These *Allium* vegetables are economically important and widely consumed in specific regions of the world, such as chives in Asia, and shallot in parts of Europe and America.

Antioxidant activity has been reported in several *Allium* vegetables, including onion (*12,13*), garlic (*5,14*), shallot (*15*), leek (*13*), chives (*15,16*) and bunching onions (*16*). However, comparisons of antioxidant strength among these species, using the same experimental conditions, have not been reported.

Previous studies have suggested that organosulfur and phenolic compounds are, in part, responsible for *Allium* antiplatelet activity. Briggs *et al*. (*9*) evaluated *in vitro* antiplatelet activity of four thiosulfinates (TSs) and found that all of them inhibited platelet aggregation, but their antiplatelet strength differed. In addition, combinations of different TSs revealed non-additive effects on *in vitro* antiplatelet activity, suggesting that the antiplatelet potential of *Allium* extracts cannot be predicted only by the presence and the treated dose of organosulfur components. Among the phenolic compounds, quercetin, the main polyphenol found in onions and shallots (*17*), inhibited platelet aggregation *in vivo* (*18*) and *in vitro* (*19*). The effect of other *Allium* phenolic compounds on platelet aggregation has not been reported to date.

Phenolic compounds have also been associated with *Allium* antioxidant activity. Total phenolic content was significantly and positively correlated with antioxidant activity in onion and shallot (*13*), garlic (*14*), and leek (*20*). Among *Allium* polyphenols, the flavonoid quercetin was further investigated, revealing strong antioxidant activity (*21*). Interestingly, quercetin is absent in garlic (*22*), which has been found to possess stronger antioxidant activity than onion (*23*), suggesting that compounds other than quercetin must be involved in garlic, and perhaps other alliums, antioxidant activity. Relationships between different phenolic compounds present in the main *Allium* vegetables and their antioxidant activity may shed light on the compound relative contribution to *Allium* antioxidant activity. High-performance liquid chromatography (HPLC) analysis is an ideal technique for this purpose, since it allows precise quantitative determination of several individual phenolic compounds simultaneously. In contrast, most of the previous *Allium* studies have used spectrophotometry-based determinations of total phenolics to establish associations between total phenolic content and antioxidant activity.

The goals of the present study are: (*i*) to perform comparative analyses of antiplatelet and antioxidant activities among the main *Allium* vegetable species, (*ii*) to investigate the antiplatelet activity of the so far uncharacterised *Allium* vegetables, (*iii*) to characterise the content of bioactive organosulfur and phenolic compounds in these species, and examine possible associations between these compounds and the functional properties.

The comparative compositional and functional characterisation of *Allium* species will provide objective information for promoting the consumption of some *Allium* vegetables on the basis of their nutraceutical value. Also, the association between individual compounds and the antiplatelet or antioxidant activities may provide rationale for further research aiming at establishing causative effects for selected compounds, for example, by testing antiplatelet or antioxidant activities in selected phenolics and estimating their relative contribution to the overall activity. Such information would be useful for breeding purposes, *e.g.* if compounds with large contribution to the trait were revealed, since rapid determinations of such compounds could be used as markers for the indirect selection of plants with high functional value.

Materials and Methods

Plant materials

Six *Allium* (Alliaceae) species were obtained from a vegetable market in Mendoza, Argentina. All the vegetables were produced locally and they appeared fresh, with no sign of oxidation or dehydration. For each species, three replicates were used. Each replicate consisted of 10 onion bulbs (mean *m*(bulb)=85 g), 10 shallot bulbs (mean *m*(shallot bulb)=40 g), 10 garlic cloves (one clove per bulb from 10 different bulbs; mean *m*(clove)=2.5 g), 10 units of leek (white shaft) and 10 and 20 g of the edible tissues of chives (leaves) and bunching onion (leaves and white shaft), respectively.

Processing of samples

Sample processing and preparation of aqueous extracts were performed as previously described by Galmarini *et al*. (*7*). Briefly, the bulb and clove outer dry scales of onion, garlic and shallot were removed and they were cut in quarters longitudinally. The edible part of chives, bunching onions and leeks was cut in fine slices. Peeled bulbs and chopped fresh tissues of each *Allium* species were juiced in two volumes (by mass per volume) of distilled water, with the exception of chives, which was juiced in four volumes of distilled water, using a blender (model MR 400 Plus; Braun, Kronberg, Germany).

In vitro antiplatelet activity

In vitro antiplatelet activity was determined in *Allium* extracts (without further dilution) as described previously for garlic (*4*) and onion (*7*) using an electrical impedance aggregometer (Chrono-Log, Havertown, PA, USA) in whole blood (*24*). Blood was collected from two healthy non-smoker donors, a male and a female, aged 30 and 25, respectively, who had abstained from eating alliums or other known platelet-inhibitory foods for at least 5 days prior to venipuncture. For each *Allium* juice sample, three technical replicates were used (*i.e.* three determinations of *in vitro* antiplatelet activity per sample). *In vitro* antiplatelet activity was expressed as percentage of platelet aggregation inhibition compared to control samples prepared in the same way but without the addition of *Allium* juice. For each species, the juice concentration required to inhibit platelet aggregation by 50 % (IC_{50}) was estimated from dose-response curves constructed using different dosages of *Allium* juice *vs.* the percentage of aggregation inhibition. IC_{50} values were expressed in mg of fresh tissue per mL of whole blood.

Antioxidant activity

Antioxidant activity in *Allium* extracts was determined based on their oxygen radical absorbance capacity (ORAC) (*25*). For this purpose, the extracts were diluted 500× in 75 mM potassium phosphate buffer (pH=7.0), and antioxidant activity was estimated using a microplate fluorometer (Fluoroskan Ascent™ FL; Thermo Fisher Scientific, Wilmington, DE, USA) according to Berli *et al*. (*26*). Antioxidant activity was expressed in μmol of Trolox equivalents (TE) per 100 g of fresh mass (fm). Two technical replicates were used for each *Allium* juice sample.

Soluble and total solids

Soluble solid (SS) content was determined with a refractometer (model ZGRC-200ATC; Científica Schonfel, Buenos Aires, Argentina) as previously described (*7*), and expressed in °Brix. For determination of total solids, expressed in dry matter (dm) content, *Allium* fresh tissues were weighed and dried in a stove at 60 °C until constant mass was obtained, and expressed in percentage (%) of fm.

Pyruvate analysis

Pyruvate content was used as an estimator of *Allium* total organosulfur content (*6*). Pyruvate determinations were performed in a Beckman DU-530 UV-Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, USA) as reported by Schwimmer and Weston (*27*) and expressed in mmol of pyruvate per 100 g of fm.

Total phenolics

Total phenolic content was estimated as described previously by Singleton and Rossi (*28*) using a Beckman DU-530 UV-Vis spectrophotometer (Beckman Coulter), and expressed in mg of gallic acid equivalents (GAE) per 100 g of fm.

HPLC analysis of phenolic compounds

The mass fraction of 11 phenolic compounds was estimated in all *Allium* species by high-performance liquid chromatography (HPLC) using an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler (WPALS G1367A), binary pump (G1312A), degasser unit (G1397A), autosampler thermostat (ALS Therm G1330B) and column compartment (COLCOM G1361A). The analytical column was an ODS-Hypersil™ C18 column (250 mm×4.6 mm, particle size 5 μm; Thermo Fisher Scientific Inc, Waltham, MA, USA). Detection of flavan-3-ols was performed with an Agilent 1100 fluorescence detector (G1321A) at λ=280 (excitation) and 310 nm (emission) and of flavonols with an Agilent 1200 diode array and multiple wavelength detector (G1365B) at λ=380 nm. The mobile phase used for separation was a linear gradient of 0.1 % trifluoroacetic acid and acetonitrile/0.1 % trifluoroacetic acid in a ratio of 80:20 (by volume). Calibration curves constructed from pure compounds of catechin aglicon, epigallocatechin gallate (EGCg), epicatechin gallate (ECg), epigallocatechin (EGC), rutin, myricetin aglicon, quercetin aglicon, kaempferol aglicon, chlorogenic acid, coumaric acid, ferulic acid and caffeic acid were used to estimate compound mass fraction. Quercetin, myricetin, catechin, kaempferol and ferulic acid glycosides were evaluated after chemical hydrolysis. The mass fraction of individual phenolic compounds was expressed in mg per 100 g of fm. ECg and EGC are reported together as ECg+EGC.

Total flavonoids and total non-flavonoid phenolics

Total flavonoid (TF) content was calculated from HPLC data as the sum of the individual flavonoid compounds present in each *Allium* species (*i.e.* TF=quercetin+catechin+EGCg+ECg+EGC+rutin+myricetin+kaempferol) and expressed in mg per 100 g of fm. Total non-flavonoid phenolic (TNFP) content was calculated from HPLC data as the sum of the individual non-flavonoid phenolics present in each *Allium* species (*i.e*. chlorogenic acid+coumaric acid+ferulic acid+caffeic acid) and expressed in mg per 100 g fm.

Thiosulfinate analysis

Thiosulfinate content was determined spectrophotometrically using a Beckman DU-530 UV-Vis spectrophotometer (Beckman Coulter) according to Han *et al.* (*29*), and expressed in mmol TS per 100 g fm.

Statistical analyses

Single-factor and multifactor analyses of variance (ANOVA), mean value comparisons, Pearson's correlation analysis and principal component analysis (PCA) were performed using InfoStat software v. 4.0 for Windows (*30*). Mean value comparisons were performed by the least significant difference (Fisher's LSD) test, and p-value<0.05 was considered significant.

Results and Discussion

Variation in antiplatelet activity among Allium vegetables

The *in vitro* responses of human platelets to dosages of six *Allium* vegetables were measured and compared. All *Allium* species exhibited significant antiaggregatory

effects, as compared to their negative controls. Antiplatelet activity varied significantly and broadly (more than 30-fold) among the six analysed *Allium* species, as indicated by their IC_{50} values (Table 1). In both blood donors, garlic was the most potent platelet inhibitor, followed by shallot and chives, whereas leek and bunching onion were the weakest antiplatelet agents. Garlic antiplatelet activity was approx. 5-, 13-, 23-, 31- and 32-fold higher than of shallot, chives, onion, bunching onion and leek, respectively, in both blood samples (Table 1).

In vitro antiplatelet activity of all the species varied in a dose-dependent manner (data not presented). Extracts of garlic and shallot inhibited platelet aggregation at all tested doses ($p<0.05$), beginning at 5 and 15–50 μL per mL of blood, respectively. The minimum platelet inhibitory doses of other *Allium* extracts were 40- to 60-fold higher (*i.e*. 200–300 μL per mL of blood) than of garlic.

Antiplatelet activity of chives and shallot has not been investigated previously. In the present study, we report for the first time a significant *in vitro* antiplatelet activity induced by aqueous extracts of these two *Allium* vegetables. Our comparative analysis revealed that shallot and chives are, after garlic, potent antiaggregant agents (Table 1).

Although experimental conditions varied greatly among previously reported studies, and therefore results cannot be directly compared, the IC_{50} values presented in this study of garlic and onion are in general agreement with those from a previous report of Ali *et al*. (*31*) using platelet-rich plasma (PRP) instead of whole blood for assessing *in vitro* aggregometry. In their study, antiplatelet activity of the two species varied approx. 16-fold, with mean IC_{50} values of garlic and onion of 6.6 and 90 mg/mL of PRP, respectively, whereas in the present study garlic antiplatelet activity was approx. 22-fold higher than onion antiplatelet activity, with mean IC_{50} values of 3.6 and 81.7 mg fm per mL of whole blood, respectively (Table 1).

Antiplatelet activities reported herein of garlic and onion are also comparable with values found for both species in our previous studies using the same *in vitro* antiplatelet activity procedure (*4*,*7*,*8*,*32*,*33*). In these studies, garlic *in vitro* antiplatelet activity (*4*,*32*) was much stronger than that of onion (*7*,*8*,*33*), on fresh mass basis. Such differences in *in vitro* antiplatelet activity between the two species are likely due to their compositional variation, and their bulb water content. Garlic has higher content of total organosulfur compounds (Table 1) and different profile of these sulfur constituents, allicin being its predominant thiosulfinate (accounting for approx. 60–95 % of total thiosulfinates in fresh garlic) (*5*). Allicin, which is absent in onion, was found to be the most potent antiplatelet *Allium* thiosulfinate, as evaluated *in vitro* (*9*). Water content in the edible parts of the two most important *Allium* vegetables may also account for the observed differences in the amount of antiplatelet compounds (*e.g.* thiosulfinates) and therefore antiplatelet activity. While most onion cultivars have approx. 90–95 % water (by mass) in their bulbs (*7*,*33*), garlic usually has water content in the range of 55–65 % (*33*). Thus, on fresh mass basis, the antiplatelet compounds in onion are much more diluted than in garlic, and this difference likely reflects part of the observed variation in *in vitro* antiplatelet activity between the two species. Presumably, and more broadly, the observed variation in *in vitro* antiplatelet activity among the six analysed *Allium* vegetables may be due to the species differences in water content in their edible parts, the type and content of antiplatelet compounds (*e.g.* thiosulfinates and phenolic compounds) and their interactions.

Although in the present study no 'donor × *in vitro* antiplatelet activity' interaction was found, significant differences in platelet aggregation by onion and shallot were observed between blood donors (Table 1). *In vitro* antiplatelet activity induced by extracts of onion and shallot was weaker in the blood of donor 2 than of donor 1. Variation in the *in vitro* antiplatelet activity among blood donors induced by onion extracts has been reported previously (*7*).

Variation of antioxidant activity among Allium species

The present study constitutes the first comparative analysis of antioxidant activity among the main *Allium* vegetable species using the same experimental conditions (ORAC). Antioxidant activity was found in all *Allium* vegetables, with a significant variation among the species. Antioxidant activity mean values varied more than 8-fold and ranged from 247.5 (in leek) to 2088.2 μmol TE per 100 g fm (in chives) (Table 1). The decreasing order for antioxidant strength was chives>shallot>garlic>onion>bunching onion>leek. Chives were significantly more potent antioxidants than all the other *Allium* species ($p<0.05$), with antioxidant activity values 5.8- to 8.4-fold higher than the weakest antioxidants; onion, bunching onion and leek (Table 1).

Our results are in accordance with a previous study by Morales-Soto *et al.* (*34*) reporting antioxidant ranges (as Trolox equivalents) on dry mass basis of 830–4300 and 520–13 000 μmol/100 g for garlic and onion, respectively. For leek, ORAC ranged from 27 to 88 μmol per 100 g, whereas in bunching onions values of approx. 50 μmol per g were reported (*35*). The ranges of antioxidant activity previously reported for leek and bunching onion are slightly higher than the values found in these species in the present study. These discrepancies could be due to the compositional differences among the assayed cultivars, water content and agricultural practices used for growing the plant materials.

Relationships between Allium functional properties and their bioactive compound content

Data from compositional analysis of edible parts of six *Allium* vegetables are presented in Table 1. In order to explore relationships between the content of bioactive compounds and *Allium* antioxidant and antiplatelet activities, correlation analyses were performed among *in vitro* antiplatelet activity, antioxidant activity and phytochemical mass fraction (Tables 2 and 3). Significantly positive correlations were found between *in vitro* antiplatelet activity and pyruvate ($R=0.74$), total phenolics ($R=0.73$) and thiosulfinates ($R=0.60$), whereas no association was found between the antiplatelet activity and total non-flavonoid phenolics (TNFP) (Table 2).

The differences found in the antiaggregatory activity are likely due to compositional variations in antiplatelet

Table 1. Antiplatelet and antioxidant properties and content of bioactive compounds in six Allium vegetable species

Species	*in vitro* APA 1 IC_{50}/(mg/mL)	*in vitro* APA 2 IC_{50}/(mg/mL)	Mean *in vitro* APA	*b*(AOA) μmol/100 g fm	*b*(pyruvate) mmol/100 g fm	*b*(thiosulfinate) mmol/100 g fm	*w*(TP) mg/100 g fm	*b*(TF) mmol/100 g fm	*w*(TNFP) mg/100 g fm	Soluble solids °Brix	*w*(dry matter) %
Garlic	(3.2±0.9)^{c}	(4.0±1.1)^{d}	3.6	(1343.5±17.7)^{c}	(3.05±0.01)^{a}	(5.9±0.2)^{a}	(78.9±11.8)^{b}	(10.8±0.6)^{de}	(4.0±0.4)^{b}	(30.3±1.5)^{a}	(29.9±0.9)^{a}
Shallot	(6.9±0.5)^{c}	(30.9±4.2)^{c}	18.9	(1527.7±121.6)^{b}	(1.8±0.1)^{c}	(0.290±0.004)^{cd}	(130.2±19.5)^{a}	(142.4±13.9)^{a}	n.d.	(15.0±0.1)^{b}	(15.4±1.1)^{b}
Chives	(45.4±0.4)^{b}	(50.1±1.0)^{b}	47.8	(2088.2±15.9)^{a}	(2.0±0.2)^{b}	(0.43±0.01)^{b}	(122.4±24.6)^{a}	(64.7±4.2)^{b}	(12.3±1.1)^{a}	(6.000±0.001)^{e}	(16.4±0.5)^{b}
Onion	(46.7±12.2)^{b}	(116.7±1.0)^{a}	81.7	(361.8±147.9)^{d}	(0.53±0.04)^{f}	(0.04±0.02)^{e}	(22.2±7.7)^{c}	(26.2±1.0)^{c}	(0.24±0.01)^{d}	(8.1±0.6)^{d}	(7.8±0.2)^{c}
Bunching onion	(113.8±2.5)^{a}	(113.2±2.2)^{a}	113.5	(294.9±77.8)^{d}	(1.04±0.02)^{e}	(0.20±0.04)^{d}	(25.8±4.0)^{c}	(16.7±1.2)^{cd}	(1.3±0.2)^{c}	(10.7±1.3)^{c}	(12.8±6.0)^{b}
Leek	(114.9±3.3)^{a}	(117.3±7.4)^{a}	116.1	(247.5±16.7)^{d}	(1.3±0.1)^{d}	(0.33±0.01)^{bc}	(26.3±3.0)^{c}	(0.94±0.01)^{e}	(2.0±0.1)^{c}	(8.7±0.3)^{cd}	(15.7±2.3)^{b}

Species	*w*(flavonoids) mg/100 g fm							*w*(non-flavonoids) mg/100 g fm			
	EGCg	EGC+ECg	Rutin	Quercetin	Kaempferol	Catechin	Myricetin	Chlorogenic acid	Coumaric acid	Ferulic acid	Caffeic acid
Garlic	n.d.	(9.1±0.6)^{b}	n.d.	n.d.	(0.5±0.1)^{c}	(1.15±0.02)^{b}	n.d.	n.d.	(0.22±0.01)^{bc}	(2.0±0.2)^{b}	(1.8±0.1)^{a}
Shallot	(14.8±2.9)^{a}	(22.2±4.4)^{a}	n.d.	(52.1±7.6)^{a}	(2.9±0.3)^{b}	(0.4±0.1)^{c}	(50.0±10.9)^{a}	n.d.	n.d.	n.d.	n.d.
Chives	n.d.	(24.8±3.0)^{a}	(1.4±0.1)^{b}	(3.9±0.4)^{bc}	(15.2±2.9)^{a}	(1.4±0.1)^{a}	(18.0±1.6)^{b}	(1.1±0.1)^{a}	(0.4±0.1)^{a}	(10.6±1.2)^{a}	(0.18±0.01)^{b}
Onion	(8.0±0.7)^{b}	(4.5±0.7)^{c}	(0.2±0.1)^{c}	(8.5±2.3)^{b}	(1.1±0.2)^{b}	(0.180±0.004)^{d}	(3.8±0.1)^{c}	n.d.	(0.01±0.01)^{d}	(0.200±0.001)^{d}	n.d.
Bunching onion	n.d.	(5.320±0.002)^{bc}	(8.7±0.9)^{a}	(0.19±0.04)^{c}	(2.0±0.4)^{b}	(0.44±0.04)^{c}	n.d.	(0.31±0.08)^{b}	(0.20±0.01)^{c}	(0.8±0.1)^{c}	n.d.
Leek	n.d.	n.d.	n.d.	(0.8±0.01)^{c}	(0.300±0.001)^{c}	(0.24±0.02)^{d}	(0.32±0.01)^{d}	n.d.	(0.29±0.05)^{b}	(1.6±0.1)^{bc}	(0.09±0.02)^{b}

Data are mean value±standard deviation (S.D.). For each variable, different letters indicate significantly different mean values according to Fisher's test ($p<0.05$). *in vitro* APA 1 and 2=*in vitro* antiplatelet activity for both blood donors respectively, as IC_{50} (mg fm/mL whole blood), AOA=antioxidant activity as Trolox equivalents, TP=total phenolics as gallic acid equivalents, TF=total flavonoids, TNFP=total non-flavonoid phenols, EGCg=epigallocatechin gallate, EGC+ECg=epigallocatechin+epicatechin gallate, n.d.=not detected

Table 2. Pairwise correlation values (R) among pyruvate, total phenols, soluble solids, dry matter, thiosulfinates, total flavonoids, total non-flavonoid phenols (TNFP), antioxidant activity and *in vitro* antiplatelet activity

	Total phenols	Soluble solids	Dry matter	Thiosulfinates	Antioxidant activity	*in vitro* APA 1 (IC_{50})	*in vitro* APA 2 (IC_{50})	Mean *in vitro* APA	Total flavonoids	TNFP
Pyruvate	0.57*	0.77**	0.95**	0.83**	0.67**	0.58*	0.89**	0.74	0.09	0.43
Total phenols		0.20	0.35	0.14	0.91**	0.68**	0.78**	0.73	0.77**	0.49*
Soluble solids			0.84**	0.93**	0.22	0.59*	0.83**	0.78	–0.07	–0.15
Dry matter				0.91**	0.44	0.47*	0.78**	0.63	–0.12	0.28
Thiosulfinates					0.27	0.51*	0.69**	0.60	–0.29	0.11
Antioxidant activity						0.70**	0.82**	0.76	0.60*	0.69**
in vitro APA 1 (IC_{50})							0.84**		0.55*	0.11
in vitro APA 2 (IC_{50})									0.45	0.33

*p<0.05 and **p<0.01; *in vitro* APA 1 and 2=*in vitro* antiplatelet activity for donor 1 and donor 2 respectively

Table 3. Pairwise correlation values (R) among *Allium in vitro* antiplatelet and antioxidant activities and the content of eleven phenolic compounds

	EGCg	EGC+ECg	Rutin	Quercetin	Kaempferol	Catechin	Myricetin	Chlorogenic acid	Coumaric acid	Caffeic acid	Ferulic acid
Antioxidant activity	0.13	0.91**	0.33	0.33	0.71**	0.80**	0.55**	0.57*	0.32	0.28	0.67**
in vitro APA 1 (IC_{50})	0.49*	0.59*	0.57*	0.51*	0.13	0.41	0.50	0.07	0.31	0.50*	0.06
in vitro APA 2 (IC_{50})	0.16	0.64**	0.38	0.35	0.23	0.67**	0.43	0.10	0.07	0.68**	0.25

*p<0.05 and **p<0.01; *in vitro* APA 1 and 2=*in vitro* antiplatelet activity for donor 1 and donor 2 respectively, EGCg=epigallocatechin gallate, EGC+ECg=epigallocatechin+epicatechin gallate

compounds among the examined *Allium* samples. *Allium* species differ in their organosulfur profiles, both in content and compound type, and isolated thiosulfinates from different alliums have proved different *in vitro* antiplatelet activity (*9*). In the present study, garlic was the most potent anti-aggregant agent, with *in vitro* antiplatelet activity values 6- to 40-fold higher than any other *Allium* species. Also, garlic had significantly higher pyruvate (an estimator of total organosulfur compounds) and thiosulfinate content than the other species. These data, together with the positive correlations found between *in vitro* antiplatelet activity and pyruvate (R=0.74), and *in vitro* antiplatelet activity and thiosulfinates (R=0.60) suggest that organosulfur compounds are involved in *Allium in vitro* antiplatelet activity.

Antiplatelet activity of phenolic compounds, such as some flavonoids, has also been reported (*18*). In this study significant positive correlations between *in vitro* antiplatelet activity and total phenolics (R=0.78), and between *in vitro* antiplatelet activity and six individual polyphenols (R=0.49–0.68) were found, suggesting that phenolic compounds are also involved in *Allium* antiplatelet activity. The strongest associations were found with caffeic acid (R=0.68), catechin (R=0.67), and EGC+ECg (R=0.59–0.64) (Table 3). Altogether, our data suggest that both organosulfur and phenolic compounds are involved in *Allium in vitro* antiplatelet activity. In addition, it is possible that interactions among some of these compounds may play an important role in determining the overall antiplatelet activity of each species.

Allium antioxidant activity was strongly and positively correlated with total phenolics (R=0.91, $p<0.001$), suggesting that phenolic compounds are extensively involved in *Allium* antioxidant properties. Similarly, antioxidant activity was also positively associated with total phenolics (R=0.60) and TNFP (R=0.69) (Tables 2 and 3). These results are in accordance with previous studies in onion and shallot (*13*), garlic (*14*), and leek (*20*), reporting significantly positive correlations between total phenolic content and antioxidant activity.

Further association analyses among individual polyphenols and antioxidant activity were performed. Significant positive correlations were found between antioxidant activity and EGC+ECg (R=0.91), catechin (R=0.80), kaempferol (R=0.71), ferulic acid (R=0.67), chlorogenic acid (R=0.57) and myricetin (R=0.55). Chives, the most potent antioxidant species, had significantly higher values of EGC+ECg, catechin, kaempferol, ferulic acid and chlorogenic acid than all other *Allium* vegetables (Table 1).

Although the most potent antioxidant species were chives and shallot, their phenolic profiles varied greatly (Table 1), suggesting that individual phenolic compounds differ in antioxidant strength. Balasundram *et al.* (*35*) proposed that the antioxidant activity of phenolic compounds depends on their chemical structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings. Our results suggest that antioxidant activity in *Allium* depends not only on the chemical structure, but also on the mass fraction of each phenolic compound and on the combinations and interactions among them. Future studies regarding the antioxidant effects of individual phenolic compounds and of their interactions would be very useful to clarify the individual contribution of each compound to the overall antioxidant activity in each species.

In addition to the apparent role of polyphenols in *Allium* antioxidant activity, we found significant correlation between the antioxidant activity and pyruvate (R=0.67) (Table 2), suggesting that organosulfur compounds may also contribute to this nutraceutical property. However, no significant correlation was found between the antioxidant activity and thiosulfinate content. These results contradict those of Block (*5*) reporting that allicin and other thiosulfinate derivatives have antioxidant properties. Further studies aiming at elucidating the contribution of thiosulfinates in *Allium* antioxidant activity are needed.

Principal component analysis

Principal component analysis (PCA) was applied on the whole data set of six *Allium* species. The dimensionality of the data was reduced from 8 partially correlated variables to 2 uncorrelated principal components (PC), PC1 and PC2, accounting for 84.2 % of the observed variation. The loadings, eigenvalues and percentage of cumulative variance are presented in Table 4. PC1 correlates

Table 4. Loadings, eigenvalues and percentage of cumulative variance for the first two principal components of the whole data set (above) for six *Allium* species and the relative proportions of phenolic compounds (below)

Variable	PC1	PC2
in vitro APA 2	0.41	–0.13
Pyruvate	–0.39	–0.09
Dry matter	–0.35	–0.29
Antioxidant activity	–0.34	0.47
in vitro APA 1	0.33	–0.21
Total phenolics	–0.32	0.53
Thiosulfinates	–0.31	–0.43
Soluble solids	–0.30	–0.40
Total flavonoids	–0.15	0.50
Total non-flavonoid phenols	–0.17	0.20
Cumulative variance/%	58.9	84.2
Variable	PC1	PC2
Quercetin	0.45	–0.19
Catechin	–0.43	–0.24
EGCg	0.37	–0.17
Ferulic acid	–0.34	–0.26
Myricetin	0.33	–0.12
Coumaric acid	–0.31	–0.28
Caffeic acid	–0.30	–0.21
Kaempferol	–0.16	0.34
EGC+ECg	–0.14	0.17
Rutin	–0.07	0.46
Chlorogenic acid	–0.07	0.56
Cumulative variance/%	39.3	66.2

PC1=principal component 1, PC2=principal component 2, *in vitro* APA 1 and 2=*in vitro* antiplatelet activity for donor 1 and donor 2, EGCg=epigallocatechin gallate, EGC+ECg=epigallocatechin+ epicatechin gallate

positively with *in vitro* antiplatelet activity and negatively with the following variables, in decreasing order: pyruvate, dry matter, antioxidant capacity, total phenols, thiosulfinates, soluble solids, and total flavonoids. PC2 correlates with total phenolic compounds, total flavonoids and antioxidant activity. The variation of the data is explained mainly by *in vitro* antiplatelet activity, pyruvate, dry matter, thiosulfinates and soluble solid content, and to a lesser extent by total phenolics and antioxidant activity. The graphic representation of the scores and loadings is presented in Fig. 1a, revealing a clear separation of the analysed *Allium* vegetables. Garlic is located in the bottom left side of the plot, which is characterised by high contents of pyruvate, solids and thiosulfinates, and low values of IC_{50} (*i.e.* lower IC_{50} values indicate higher *in vitro* antiplatelet activity). Onion, leek and bunching onion are located in the right bottom side, presenting high IC_{50} and low pyruvate, soluble solids, dry matter and thiosulfinate values. Shallot and chives were clearly differentiated from the other species by their high antioxidant capacity and total phenolic content. The PCA illustrated the strong correlations observed between antioxidant activity and total phenolics, and between *in vitro* antiplatelet activity and pyruvate, soluble solids, dry matter and thiosulfinates (Table 2). Concordantly, the fact that garlic and chives, the most potent antiplatelet and antioxidant species respectively, are situated in opposite quadrants of the plot suggests that organosulfur and phenolic compounds contribute different to *Allium* antiplatelet and antioxidant activities.

Fig. 1b presents PCA of phenolic profiles of the six *Allium* species. Eleven partially correlated variables were reduced to 2 uncorrelated principal components, PC1 and PC2, accounting for 66 % of the variation. PC1 mainly correlates with quercetin, catechin, EGCg, ferulic acid and myricetin. PC2 correlates with chlorogenic acid, rutin and kaempferol. In general, the PCA revealed a clear separation of the species according to their phenolic profiles. A clear separation of the studied species was observed. Shallot and onion were located in the bottom right side of the plot, which is characterised by high quercetin and EGCg content, whereas garlic and leek were in the bottom left side, characterised by high levels of catechin. Chives and bunching onion were located in the upper side of the plot, characterised by high mass fraction of chlorogenic acid, rutin and kaempferol.

Conclusions

Under our experimental conditions garlic and shallot were the most potent antiplatelet agents, consistently in the blood of both donors, whereas chives and shallot had the strongest antioxidant activities. Leek, bunching onion and onion were, in that order, the weakest antiplatelet and antioxidant agents. The present study has character-

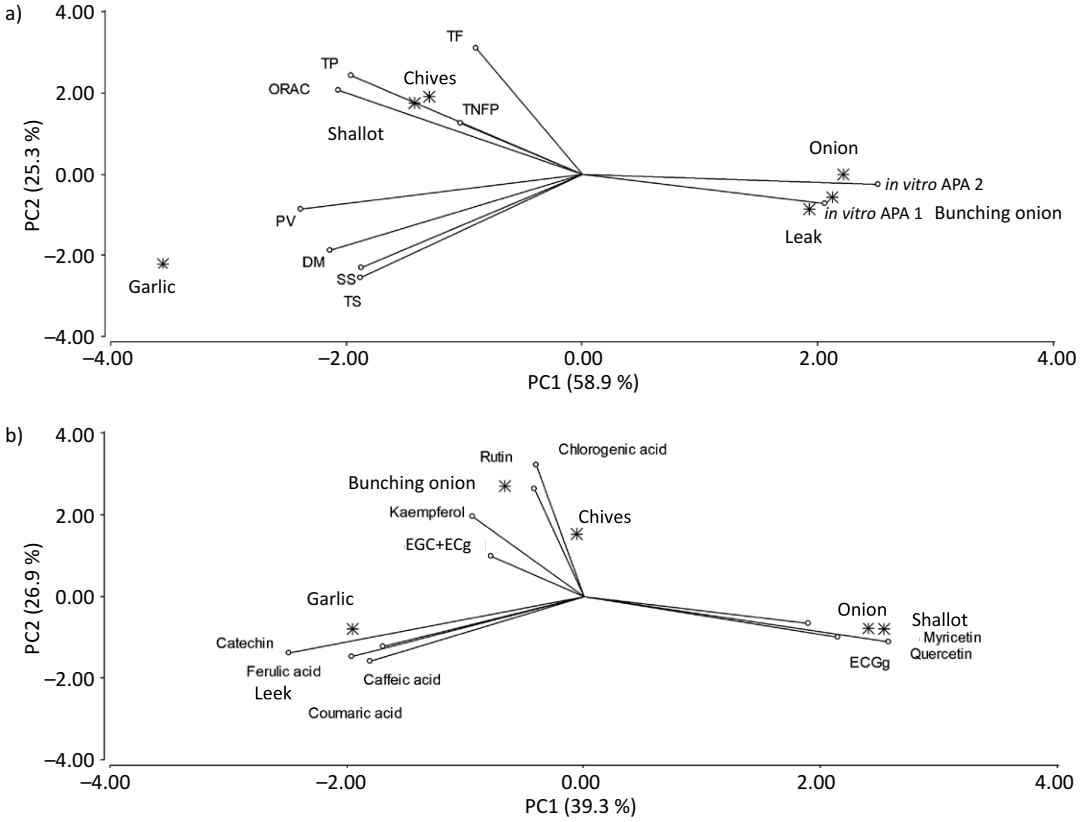

Fig. 1. Principal component analysis (PCA) of *Allium* functional properties and phytochemical content: a) PCA included the following variables: pyruvate (PV), total phenolics (TP), soluble solids (SS), dry matter (DM), thiosulfinates (TS), total flavonoids (TF), total non-flavonoid phenolics (TNFP), antioxidant activity measured by the oxygen radical absorbance capacity (ORAC), *in vitro* antiplatelet activity (APA) of both donors expressed as IC_{50} 1 and 2, respectively; b) PCA of the phenolic profiles expressed as relative proportion (%). EGCg=epigallocatechin gallate, EGC+ECg=epigallocatechin+epicatechin gallate

ised for the first time *in vitro* antiplatelet activity of chives and shallot. Interestingly, shallot was one of the most potent species with both biological activities (antiplatelet and antioxidant activities).

Our results of correlation analyses strongly suggest that organosulfur and phenolic compounds are involved, to different extents, in both functional properties. While organosulfur and phenolic compounds contribute to similar extents in *Allium in vitro* antiplatelet activity, phenolics as a whole were largely responsible for antioxidant activity, with catechin, epigallocatechin and epicatechin gallate being the individual phenolics with strongest association with *Allium* antioxidant activity.

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