Flavonoid-Metallic Cation Interactions Studied by Antioxidant Potential of Mixtures in Methanolic Solution

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Abstract

Flavonoid-metal cation interactions were studied via antioxidant property evaluation with binary mixtures of 08 flavonoids with 10 metal cations at different concentrations. We have shown in the analysis by DPPH· Radical method, that despite different behaviors of metals, antioxidant capacity is reduced for some mixtures in methanolic solution, ratio 1:1 of flavonoids: quer-cetin, rutin, luteolin-7-glucoside and eriodictyol with Al^{3+}, Fe^{2+} and Fe^{3+} ions. After analysis of influences of concentrations, studied metallic cations were divided in three groups. These cations also lead to an improvement in dose-dependent antioxidant capacity of flavonoids, and this more for aglycone compound than for glycosides. These observed interactions could well exist in plant extracts rich in major cations co-extracted and may be assessed in global evaluation of antioxidant or biological properties.

Keywords: Chelation; Flavonoid; Inorganic cation; Antioxidant capacity

Introduction

Flavonoids, an important group of polyphenols in plants are targeted for medicinal, alimentary or biocidal applications due to their antioxidant activity [1,2]. These phytomolecules can also chelate metal ions [3]. Many studies on quercetin, rutin or myricetin complexes with cations such as Fe^{2+}, Fe^{3+} and Cu^{2+} are reported in literature [3-6]. This complexation could increase antioxidant capacity of flavonoid compared to free compound [7-10], and thus affects biological properties [3,11,12]. The present study aims to detect signs of flavonoid-metallic cation interactions by monitoring antioxidant activities of salts, flavonoids and mixtures: flavonoid-salt. For that 08 flavonoids representing three subclasses (flavonols, flavones and flavanones) and a series of 10 metal cations: Ca^{2+}, Na^{+}, Al^{3+}, Pb^{2+}, Ba^{2+}, Ag^{+}, Ni^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} at different concentrations were used. The simple, rapid, inexpensive DPPH method was used to evaluate the antioxidant capacity of flavonoids alone in methanol or in presence of inorganic ions [13].

Materials and Methods

The flavonoid' standards purchased with purity between 94 to 99%: quercetin dihydrate, rutin hydrate, luteolin-7-glucoside, apigenin, hesperidin, hesperidin, eriodictyol and naringin hydrate were used. The inorganic salts used were over 95% of purity: Magnesium sulfate ($MgSO_4$), sodium nitrate ($NaNO_3$), calcium chloride ($CaCl_2$), barium chloride dihydrate ($BaCl_2$, $2H_2O$), aluminum chloride hexahydrate ($AlCl_3$, 6 H_2O), silver nitrate ($AgNO_3$), nickel sulfate ($NiSO_4$), lead nitrate $Pb(NO_3)_2$, iron sulfate heptahydrate ($FeSO_4$, $7H_2O$), iron trichloride hexahydrate ($FeCl_3$, $6H_2O$). The reagent used for antioxidant activity evaluation was 1,1-diphenyl-2-picrylhydrazyl (DPPH). Analytical grade methanol (99.8%) was used as dissolving solvent of standards for analyses.

Preparation of methanolic solutions of flavonoids and salts

Stock methanol solutions were prepared for by dissolving appropriate amount each flavonoid in methanol at 200 µM concentration. Less soluble flavonoids in methanol: luteolin-7-glucoside, apigenin and eriodictyol, are previously dissolved in a minimum of DMSO and final volume was obtained by supplementing with methanol. Diluted solutions at 100 µM of flavonoids were prepared.

For inorganic salts, stock solutions, 10000 µM concentration and diluted solutions of 100 µM, 200 µM, 250 µM, 500 µM and 1000 µM concentrations were prepared in methanol.

The combinations: flavonoid and salt were prepared by mixing in equal volume (200 µL), diluted solution of each flavonoid with a diluted methanolic solution of salt; the final concentration becomes 50 µM for flavonoid and 50 µM, 100 µM, 250 µM or 500 µM for salt meaning resulted concentration ratios (flavonoid-salt) 1:1, 1:2, 1:5 and 1:10.

The flavonoid-salt mixtures were vortexed for 2-5 min and then incubated in dark at room temperature for 2 h to allow sufficient time for potential complexation. The undiluted flavonoid-salt mixture was used for colorimetric analysis of DPPH

assay at different concentrations of inorganic salts.

Evaluation of DPPH scavenging activity

The antioxidant capacities of solutions of flavonoid, salt and their binary mixtures were measured by DPPH method adjusted to use of microplates [14] with slight modifications. The solution of DPPH was prepared in methanol at concentration 100 µM. In an eppendorf containing 2×280 µL of freshly prepared DPPH (100 µM) solution, a volume of 2×20 µL of diluted standard (100 µM), salt or mixture solution was added. The reaction mixture was shaken for a few seconds and then incubated in dark at room temperature for 2 h. A aliquot 300 µL volume was transferred to a microplate well and absorbance was reading at 517 nm. A 300 µL of DPPH solution (100 µM) was used as blank. To approximate practical conditions of plant extracts operations, no pH adjustment using buffer solution was applied.

The percentage of DPPH· radical inhibition was calculated according to equation 1,

Inhibition (%)=$[(A_{DPPH}-A_{sample+DPPH})/A_{DPPH}]*100$ Eq. 1

where A_{DPPH} is the absorbance of DPPH solution in methanol and $A_{Sample+DPPH}$ is the absorbance of DPPH solution after reaction with standard solutions. All assays were performed in triplicate.

Statistical analysis

Data were analysed using Minttab.18 software. The analysis of variance (ANOVA) was done according to Fisher which allowed calculation of means, standard deviations and significance groupings.

Results and Discussion

Antioxidant profiles of inorganic salts

Inhibition values of DPPH· radical by salt's solutions at three concentrations in methanol: 100 µM, 200 µM and 500 µM are provided in Figure 1. The methanolic solutions of inorganic salts showed more or less pronounced inhibitory effect of DPPH Radical. The rates for concentrations 100 µM can be considered less than 10% except for sodium and barium. The highest inhibition rate is obtained with $AlCl_3$ 500 µM, i.e. 33%.

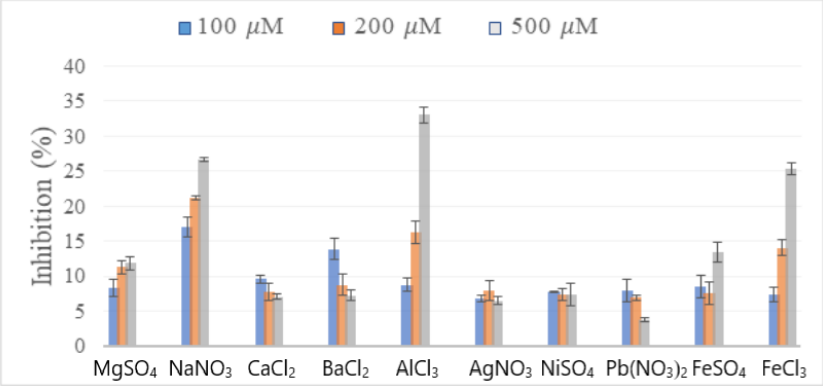

Figure 1: Histogram of DPPH inhibition rate by methanolic solutions of inorganic salts

The difference between inhibition rates for salt solution analysed at concentration 100 µM and at 500 µM, reaches maximum value with $AlCl_3$ passing from 8% to 32%. Values for inhibition percentages are increasing with ions concentration for Mg^{2+}, Na^+, Al^{3+}, Fe^{2+}(Fe II) and Fe^{3+}(Fe III). For solutions of Ba^{2+}, Pb^{2+}, Ag^+ and Ni^{2+} ions, relatively low, decreasing or constant inhibitions were observed with salt concentration. DPPH inhibition requires a transfer of electron or labile hydrogen atom that salts don't possess apart Fe^{2+} ion [15]. But the antioxidant capacity variation for Mg^{2+}, Na^+, Ca^{2+}, Fe^{2+} and Fe^{3+} solutions was already observed by Al-dabbas *et al.* [16].

We note that high inhibition rates are for Al^{3+}, Fe^{2+} and Fe^{3+} ions provided by hexa or hepta-hydrate salts ($AlCl_3$,6 H_2O; $FeSO_4$,7H_2O; $FeCl_3$,6H_2O). In addition, rate of DPPH· Radical inhibition increases with salt concentration that provides several co-crystallized water of hydration molecules per hydrated salt molecule used. The inhibitory activity of DPPH· by $AlCl_3$ (acidic salt) dissolved in water at concentration of 125 mg/ml is 17.49 ± 3.07% in evaluation of Saraç *et al.* [17] This value is similar to the obtained one for a concentrated 200 µM $AlCl_3$ solution in our study. Sodium nitrate ($NaNO_3$), presents at 100 µM concentration, the highest inhibition rate. This neutral food additive, known to be a source of membrane-destroying ROS production is hygroscopic [18]. So hydrated salts in methanol, could present strong proton donor from constitutive water which further facilitate hydrogen transfer to radical DPPH· and create conditions for expression of an antioxidant capacity.

Effect of nature of flavonoid-metal cation mixture in solution on DPPH: Radical inhibition.

The percentages of inhibition measured by DPPH method for 08 flavonoids representing three subclasses: flavonols (quercetin and rutin), flavones (apigenin and luteolin-7-glucoside) and flavanones (hesperidin, hesperetin, naringin and eriodictyol) alone are in decreasing order: Q (54%)>R(53%)>LG (53%)>Er(40%)>N (19%)>A(17%)=Ht(17%)>Hd (14%). These values and order are close to results obtained by Skroza *et al*. [19] in FRAP and DPPH tests.

Figure 2 shows values obtained in radical inhibition rate by rutin in 1:1 ratio with different metallic cations. A significant decrease in inhibitory capacity is observed with Al^{3+}, Fe^{2+} and Fe^{3+} ions and influence is said to be negative (-). It is a sign of significant ion-rutin interaction with these ions known to be active chelators. All results obtained were therefore analysed in terms of influences. The rutin mixtures with Ni^{2+}, Ca^{2+} and Na^{+} in solution seem to present strongest antioxidant capacity in binary mixtures for ratio 1:1 so higher than the one of flavonoid taken alone. In this case, influence is said to be positive (+). Calcium element as one major cation found in aqueous botanical extract could present chelation with flavonoids, with an effect on antioxidant capacity [12].

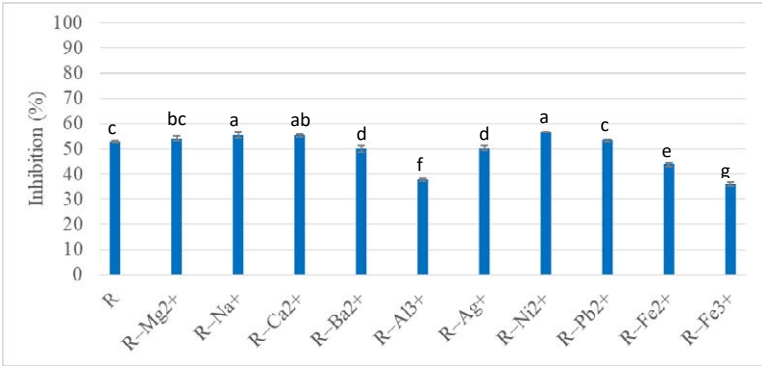

Figure 2: DPPH· radical inhibition activity by inorganic salts in 1:1 ratio with rutin in methanol

Inhibition percentages measured for binary mixtures of rutin with Al^{3+} by passing from ratio (flavonoid-ion) of 1:1 to 1:10 show antioxidant capacity with higher values from 37.91 to 53.32 respectively. To report trends observed with all binary combinations studied, some codes are used for increased influence (↑), decreased (↓) or in randomly order from ratio1:1 to ratio 1:10. All observations are presented in Table 1. The statistical analysis allows to identify type of influence according to obtained values.

Table 1: Influence of presence of inorganic ion on antioxidant capacity of flavonoid

Cation	Q^{g}	R^{c}	LG^{e}	Er^{e}	Hd^{ef}	Ht^{f}	N^{d}	A^{d}
Al^{3+}	–i↑	–f↑	–f	–f↑	+b	+d↑	+a↑	+c↑
Ag^{+}	+e	–d	+d	–f↓	+bcd↑	+de	0d	0d
Ba^{2+}	+f	–d	+a	+bc	+cde	+c	+bc	+c
Ca^{2+}	+c	+ab	+ab	+b↓	0def	+c	+b	+c
Fe^{2+}	–h	–e↑	–h↑	–h↑	0def↑	+e↑	+b↑	+c↑
Fe^{3+}	–j↑	–g↑	–g↑	–g	0f↑	+e↑	+b↑	0d↑
Mg^{2+}	+d	+bc	+d	+d	+bc	+b	+a↓	+b
Na^{+}	+b	+a	+d	+a	+a↑	+a	+a	+a
Ni^{2+}	+c	+a	+c	+cd	+bcd↑	+de	0d	0d
Pb^{2+}	+a	0c	+bc	+a↓	+cde↓	+e	0cd	0d

'+': positive influence; '-': negative influence; '0': null influence; '↑': Inhibition rate increases with ion concentration; '↓': inhibition rate decreases with ion concentration.

Q: quercetin; R: rutin; LG: luteolin-7-glucoside; Er: eriodictyol; Hd: hesperidin; Ht: hesperetin; N: naringin; A: apigenin.

One can note that influences of inorganic cations were rather positive with aglycones as hesperetin and apigenin. More negative or null influences are observed with rutin, a diglycoside derivative of quercetin (aglycone flavonol). The same observation is done for hesperetin as aglycone flavanone (exclusively in positive influence), compared to its glycosides: hesperidin and naringin. More broadly, positive influences were more observed with aglycones than with their glycosides presenting negative and even null influences. As a result, glycosylation of hydroxyls decreases chelating capacity of flavonoids and negatively influences antioxidant capacity [20].

After analysis of all influences on flavonoid antioxidant capacity, the studied inorganic ions were divided into three groups. The group 1 concerns: Mg^{2+}, Na^{+}, Ba^{2+} and Ca^{2+}, source of positive influence on 08 flavonoids in general apart from null influence of Ca^{2+} with hesperidin and negative influence of Ba^{2+} with rutin. The group 2 constituted by Ni^{2+}, Pb^{2+} and Ag^{+} ions differs from group 1 by some null influences with naringin and apigenin, and negative effect of Ag^{+} on rutin and eriodictyol.

Group 3, concerns Al^{3+}, Fe^{2+} and Fe^{3+} ions, with most negative, no and few positive influences with flavonoids. Moreover, negative influence of group 3 ions seems to be exclusively observed for flavonoids with catechol group (3′, 4′- diol) as quercetin and rutin compared to other flavonoids [5].

The decrease in antioxidant capacity of quercetin in presence of Al^{3+} ion was also revealed by Pękal *et al*. and for other ions such as Zn^{2+} and Cu^{2+} [21]. However, mixture of quercetin and Al^{3+} exhibits lower inhibitory capacity which subsequently increases also with Al^{3+} ions concentration. In sum, flavonoids with high antioxidant capacity (flavonol or aglycone) have their inhibitory capacity reduced in 1:1 ratio by presence of Al^{3+}, Fe^{2+} and Fe^{3+} cations which chelate appropriate positions of flavonoid making them unavailable for $DPPH^{\cdot}$ radical inhibition during the assay. When the ions concentration increases other sites are involved in chelation and influence the cycle stabilisation. A prepared Co(II)-quercetin complex showed higher antioxidant activity than quercetin alone by DPPH method like ions in group 1 of the present study [22]. In another study, quercetin, rutin and galangin metal complexes presented better antioxidant activity than free flavonoids. Rubens *et al.* suggest hydroxy (more acidic) 3-OH and 4-oxo groups as first binding sites. The 3′,4′-dihydroxy groups are second binding site [23,24]. Complexation stabilizes ring A, and decreases antioxidant activity, while stabilization of ring B increases antioxidant activity. So the negative influence observed on antioxidant capacity of flavonoids in mixing ratio 1:1 with Al^{3+}, Fe^{2+} and Fe^{3+} ions could be in correlation with catechol group chelation first. In literature, a chelation of Fe^{2+} or Fe^{3+} ions followed by redox reactions cited in polar solvent is proposed in particular for quercetin through catechol site with binary mixing ratio 1:1[25].

Conclusion

Without proceeding with synthesis and characterization of complexes, sign of interaction metal-flavonoid interaction were observed depending in cations ratio with effect on reducing or increasing antioxidant capacity.

Antioxidant capacity is reduced for mixtures in methanolic solution, ratio 1:1 of flavonoids: quercetin, rutin, luteolin-7-glucoside and eriodictyol with the most chelating ion Al^{3+}as Fe^{2+} and Fe^{3+} ions. These metal cations also lead to an improvement of dose-dependent antioxidant capacity of flavonoids and this more with aglycone compounds than glycosides.

The metal-flavonoid interaction would increase antioxidant capacity and affect solubility, stability in botanical formulations prepared for various applications.

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No Conflict declaration

Authors declare no conflict of interests.

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