The Equifinal Achievement of the Total Antioxidant Activity of Flavonoids by Plants in Various Habitats

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Abstract. The most important nonspecific stress-protecting function of flavonoids is antioxidant, which is associated with blocking the spread of free radicals in cells. Moreover, the effectiveness of individual classes of flavonoids as antioxidants is different; in particular, substances can differ by three or more times. The accumulation of flavonoids by individual plants is exceptionally high in plasticity. To estimate the total potential antioxidant activity, we proposed an indicator – the antioxidant status of flavonoids, taking into account the qualitative and quantitative composition of the identified flavonoids. It has been shown that relatively close values of this indicator can be formed due to different quantitative ratios of flavonoids - either a relatively large number of low-efficiency flavonoids or a much smaller number of high-efficiency ones can accumulate in plants. It has been established that this is characteristic of various species of plants that live in a variety of conditions – from horse marshes to the steppe zone and salt marshes.

Keywords: Antioxidant activity · Flavonoids · Trolox equivalents · Equifinality · Antioxidant status

1. Introduction

For plants growing in the wild, it is typical that the effects of environmental factors on them are complex and unpredictable. This is due to the high mosaic of their habitat conditions and the complex nature of fluctuations in local soil conditions in response to seasonal changes in temperature or humidification conditions. The mobility of any compound in the soil is determined by both the compound’s properties and the medium’s characteristics. It does not depend on the mobility and distribution of other compounds. Therefore, almost any combination of compounds present in a given landscape or biocenosis can be added to soils [2-11, 19-20, 31]. For example, if for each soil element, a conditional scale of only three values is adopted: “deficit-norm-toxic maximum,” then with the number of 15 factors considered, the total possible number of combinations will be 3 to 15 options.

In most cases, plants implement the principle of multiple adaptive responses, apparently as the only possible one. Its essence is as follows. In response to the environment’s requirements, the plant launches as many protective mechanisms as it can afford for resource provision. Previously, our studies have shown that the overall list of plant-triggered defensive tools, as well as the involvement of each of them in the cumulative adaptive response, can change stochastically [1, 16-18, 22, 24-30].

Flavonoids are physiologically active substances (secondary metabolites) that regulate many functions in plants and animals [21]. Flavonoids are considered essential components of the formation of plants’ nonspecific resistance to various kinds of stresses, while the composition of plant flavonoids,
like many other secondary metabolites, is characterized by significant plasticity and low predictability [12, 22-26, 29].

With respect to the accumulation of flavonoids in plants, significant intra- and interpopulation differences in the collection of these substances can be said to occur, with virtually no strong correlation between the content of individual essences [1, 16-18]. The flavonoid biosynthesis scheme is quite complicated and allows plants to widely vary the qualitative and quantitative composition, synthesize the same compound in several alternative ways, and many more.

One of the main functions of flavonoids in plants is antioxidant, protecting plants from free radicals under various stress types.

Therefore, it seems essential for us to study how the plasticity of individual flavonoids’ accumulation affects these compounds’ potential total antioxidant activity.

2. Materials and Methods

The objects were samples of individual chromatograms of extracts of aboveground parts of 8 plant species of the Urals and Western Siberia (Table 1). Samples of aboveground parts of the plants from which the quotes were obtained are collected in various populations that differ in habitat conditions.

| Species name            | Community                   | Habitat                                      |
|-------------------------|-----------------------------|----------------------------------------------|
| Atriplex patula         | Festuco-Puccinellietea      | Stepes and salt flats of the Bashkir Trans-Urals |
| Bassia seidoides        | Festuco-Puccinellietea      | Stepes and salt flats of the Bashkir Trans-Urals |
| Kochia prostrata        | Festuco-Puccinellietea      | Stepes and salt flats of the Bashkir Trans-Urals |
| Juniperus sabina        | Festuco-Brometea;           | Steppes and hills Bashkir Trans-Urals        |
|                         | Polygono-Artemisietea Austriacae |                                               |
| Glycyrrhiza korshinskii | Festuco-Puccinellietea      | Steppe and salt flats of the Bashkir Trans-Urals |
| Oxyccoccus palustris    | Oxyccocco-Sphagnetea        | Oligotrophic Swamps of Western Siberia       |
| Chamaedaphne calyculata | Oxyccocco-Sphagnetea        | Oligotrophic Swamps of Western Siberia       |
| Andromeda polifolia     | Oxyccocco-Sphagnetea        | Oligotrophic Swamps of Western Siberia       |

*Source: Compiled by the authors.*

To measure the flavonoids, alcohol extracts were chromatographed on a Luna C18 250×4.6 mm, 5 µm column in a reverse-phase system. Flavonoids were measured in the aboveground organs of licorice and juniper by HPLC. The analysis used the Sigma-Aldrich standards: baicalein, hesperetin, fisetin, naringin, naringenin, rutin, quercetin, isoquercetin, morin, dihydroquercetin, and liquiritigenin 92% minimum purity. Flavonoid standards and substances in the samples were detected at 275 and 360 nm on a diode matrix UV analyzer. Standards and importance in the specimens were detected at 275 nm.

It is customary to evaluate compounds’ antioxidant activity in individual substances’ units of action conditionally accepted standards. In Russia, the synthetic antioxidant Trolox (C14H18O4), a water-soluble analog of vitamin E, is most often used as a standard. The antioxidant activity of other compounds is expressed in units of their concentration, that is, in TEAC, (Trolox equivalent antioxidant capacity, mMol) or Trolox equivalents (Tyukkant For compounds with high antioxidant properties (mono- and especially gallates of catechins), this value can be six or more mMol of TEAS.

To assess the total antioxidant activity of flavonoids, we proposed an antioxidant status (AC) indicator. This indicator can be calculated as the sum of the products of the content of individual
flavonoids in the plant raw materials (in mg/g dry weight) by the antioxidant activity of each substance (TEAC, mMol):

\[ AC = \sum_{i=1}^{n} TEAC_i \times [\cdot]_i \]

AC – antioxidant status; TEAS – Trolox-equivalent flavonoid, mMol; \([\cdot]\) is the plant’s flavonoid content, mg/g dry weight.

Thus, it becomes possible to evaluate a flavonoid’s participation in forming total antioxidant activity in plants in specific habitats. Moreover, a separate plant, due to the polyvariant nature of its adaptive responses [24, 26], can go in different ways of flavonoid biosynthesis: from the accumulation of a small number of relative flavonoids with relatively high efficiency to biosynthesis of a large number of somewhat less effective antioxidants.

3. Results and Discussion

The participation of individual flavonoids informing the total Trolox equivalent is presented in figures 1–9. From the data presented in figures 1–9, it follows that in all studied plant species in different habitats, the ratio between the content of individual flavonoids changes and the participation of each substance in the formation of total antioxidant activity.

**Figure 1.** *Atriplex patula*; (a) concentration of flavonoids in individual plants; (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source:* Compiled by the authors.

**Figure 2.** *Bassia seidoides*; (a) content of flavonoids in individual plants (1-3, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source:* Compiled by the authors.
Figure 3. *Kochia prostrata*; (a) content of flavonoids in individual plants (1-3, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source:* Compiled by the authors.

Figure 4. *Glycyrrhiza korshinskyi*; (a) the content of flavonoids in individual plants (1-4, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source:* Compiled by the authors.

Figure 5. *Juniperus Sabina* (male plant); (a) content of flavonoids in individual plants (1-5, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source:* Compiled by the authors.
Figure 6. *Juniperus Sabina* (female plant); (a) content of flavonoids in individual plants (1-5, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source*: Compiled by the authors.

Figure 7. *Oxycoccus palustris*; (a) content of flavonoids in individual plants (1-9, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source*: Compiled by the authors.

Figure 8. *Chamaedaphne calyculata*; (a) content of flavonoids in individual plants (1-9, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source*: Compiled by the authors.
**Figure 9.** *Andromeda polifolia*: (a) content of flavonoids in individual plants (1-9, cenopopulation); (b) participation of individual flavonoids in the formation of the total Trolox equivalent. *Source:* Compiled by the authors.

**Table 2.** Ratios of variation in the content of individual flavonoids in the studied plant species.

| Species name                      | Variation coefficient, % |
|-----------------------------------|--------------------------|
| Naringin                          |                          |
| Rutin                             |                          |
| Dehydroquercetin                  |                          |
| Fisetin                           |                          |
| Merin                             |                          |
| Quercetin                         |                          |
| Hesperetin                        |                          |
| Baicalein                         |                          |
| Atriplex patula                   | 137.5                    |
| Bassia seidoides                  | 16.63                    |
| Kochia prostrata                  | 48.27                    |
| Yuniperus sabina (male plants)    | 108.31                   |
| Yuniperus sabina (female plants)  | 111.5                    |
| Glycyrrhiza korshinskii           | 81.87                    |
| Oxyccoccus palustris              | 65.31                    |
| Chamaedaphne calyculata           | 83.75                    |
| Andromeda polifolia               | 80.68                    |
| On average for the studied plant species of the Bashkir Trans-Urals | 84.01 |
| On average, for the studied plant species of oligotrophic Swamps in Western Siberia | 76.58 |

*Note:* "-" - the substance was not detected.

*Source:* Compiled by the authors.

The coefficients of variation of the content of individual flavonoids are presented in table 2. The variables of the total Trolox equivalent are presented in table 3.
Table 3. Variables of the total Trolox equivalent in the studied plant species.

| Species name                       | Total trolox-equivalent (mM TEAC) | minimum | maximum | Variation coefficient, % |
|------------------------------------|-----------------------------------|---------|---------|--------------------------|
| Atriplex patula                    | 0.69                              | 3.39    | 63.70   |                          |
| Bassia seidoides                   | 1.97                              | 8.76    | 71.36   |                          |
| Kochia prostrata                   | 8.85                              | 29.39   | 79.41   |                          |
| Yuniperus sabina (male plants)     | 25.43                             | 73.24   | 33.02   |                          |
| Yuniperus sabina (female plants)   | 31.35                             | 218.02  | 64.75   |                          |
| Glycyrrhiza korshinskii            | 8.04                              | 58.93   | 76.32   |                          |
| Oxycoccus palustris                | 197.59                            | 345.36  | 20.97   |                          |
| Chamaedaphne calyculata            | 155.89                            | 232.82  | 18.94   |                          |
| Andromeda polifolia                | 155.59                            | 292.24  | 26.17   |                          |

Source: Compiled by the authors.

Figure 10 presents the results of the cluster analysis of individual flavonoid compounds in the plants studied. Figure 11 presents the results of a cluster analysis of flavonoid content for individual plant species.

Figure 10. Cluster analysis of the content of individual flavonoids. Source: Compiled by the authors.

Figure 11. Cluster analysis of investigated plant species by the position of the content of individual flavonoids. Source: Compiled by the authors.
The observed picture seems somewhat chaotic, although one cannot fail to notice some similarities in the accumulation of flavonoids by endemic plants of the same habitats – halophytes and horseback plants. However, when it comes to achieving a single antioxidant status, a certain general logic looms (Tab. 3, Fig. 11). Flavonoids are most conservatively accumulated and used as antioxidants by plant species typical of upper oligotrophic marshes. The total antioxidant activity indicator reaches the tremendous Variability in plants – halophytes and plants adapted to the high content of salts in soils (Tab. 3). However, in general, it can be argued that the logic for forming the total antioxidant status of flavonoids is included regardless of the accumulation of individual substances – in general, the variability indicator of the antioxidant quality is more stable than the Variability of unique compounds.

This phenomenon is observed in plants in all studied habitats – from horse marshes (Oxyccocco-Sphagnetea community) to dry steppes (Festuco-Brometea and Polygono-Artemisietea Austriacae communities) and salt marshes (Festuco-Puccinellietea). For the above organizations, the studied plant species are typical, that is, these species have adapted to habitat conditions for many years. However, it can be argued that in natural habitats, due to the high level of stress, the anti-stress antioxidant role of flavonoids continues to be relevant. It was established that for the accumulation of individual flavonoids in each habitat, each plant behaves in its way. This seems to be one example of plants implementing the diversity of primary adaptive reactions and equifinality in achieving the necessary adaptive results. This phenomenon, by its nature of manifestation, has signs of similarity with a strange attractor.

4. Conclusion
The analysis of the obtained data makes it possible to draw the following conclusions:

1. The content of individually identifiable flavonoids varies at all levels: between the cenopopulations of the same species, species within the boundaries of ecosystems, and between plants of oligotrophic swamps and the trans-Ural steppes. In general, we can talk about the formation of regional complexes of flavonoids at all levels.
2. The Variability of the content of individual flavonoids can vary from 8.3 to 223.6%. In species – inhabitants of the riding swamps of Western Siberia, the accumulation of flavonoids occur, in general, more conservatively, which affects lower values of the coefficients of variation.
3. The coefficients of variation of individual flavonoids are higher than the coefficients of interpretation of the sum of flavonoids. Thus, the involvement of many flavonoids in adaptation under specific conditions leads to an increase in plants’ overall resistance and homeostatisation in fluctuating media.

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